Clinical Laboratory Science Review

a bottom line approach

Fifth Edition

by Patsy Jarreau, MHS, MLS(ASCP)
with
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A Bottom Line Approach

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We would also like to thank Sylvia Rayfield, who donated publication rights for this work to Louisiana State University Health Sciences Center in honor and memory of Betty Lynne Theriot, the original author whose professional and personal life brought tremendous enlightenment to so many of us.

We gratefully recognize the continual support of the School of Allied Health Professions and the Foundation of the LSU Health Sciences Center.

— The Department of Clinical Laboratory Sciences
LSU Health Sciences Center
New Orleans, LA

Dedication

Betty Lynne Theriot
1954-1997

This book is dedicated to Betty Lynne Theriot whose creativity and inspiration made this, the fourth edition, and the previous editions possible. As president and founder of Creative Educators, she developed instructional methods in clinical laboratory science and allied health and presented extensively at local, regional and national conferences. She was co-author and editor of several books published by Creative Educators including Clinical Laboratory Science Review: A Bottom Line Approach, Clinical Instruction in Blood Banking, and Clinical Instruction in Parasitology.

Betty Lynne was very active in professional organizations and was honored as Louisiana Society for Clinical Laboratory Science Member of the Year in 1997, Educator of the Year in 1994, and recipient of the American Society for Clinical Laboratory Science Sherwood Medical Professional Achievement Award in Education in 1997.

Her loss has had a profound effect on the profession of clinical laboratory science. She was the ultimate educator persistently seeking new information and using innovative methods to make learning fun and more effective. She stimulated creativity and was an inspiration and mentor to her students and colleagues. Her willingness to provide assistance to other professionals was recognized nationally.
Betty Lynne's energy, vitality, and infectious enthusiasm will remain forever with those who knew her.

Proceeds from the sales of this book are used to fund scholarships for students in clinical laboratory science. In 2004 the Betty Lynne Theriot Distinguished Professorship was established in the LSU Health Sciences Center Department of Clinical Laboratory Sciences. We look forward to funding more scholarships and other worthy projects to benefit the profession through continued sales of this book.

Preface

This book is intended for all students of clinical laboratory science as a review reference as well as a course supplement. It is also relevant for medical students and other allied health students who are responsible for knowledge of the many clinical laboratory tests currently performed on patients. Educators in allied health, nursing and other medical careers will benefit from the many "memory tools" which simplify educational concepts.

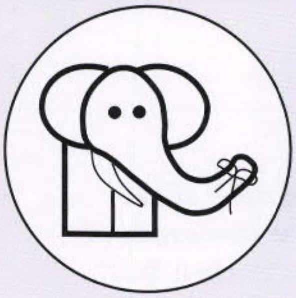
Introduction

Welcome to the unique study experience we offer in this book. Because of our clinical and academic backgrounds, we've been able to streamline the thousands of details in the clinical laboratory science profession to determine the "bottom line".

To HELP you remember this bottom line, we've created graphics, stories, acronyms and mnemonics.

Handy test alerts *(for concepts frequently tested)*
Easy format *(concise outlines, charts and cross-referenced index)*
Learning tools *(to jog your memory)*
Practice questions *(with answers and rationale)*

Good luck on the exams and in your career!



REMEMBER!

It's Not What You Know, But What You Remember That Counts!

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IMMUNOHEMATOLOGY

by Elizabeth F. Williams

Donor Selection and Blood Collection

COLLECTION OF DONOR BLOOD

1. Registration, medical history and physical examination *(See Danny Donor)*
2. Aseptic technique, scrub site for minimum 30 seconds- providine-iodine scrub

GENERAL INFORMATION

1. Maximum collection — no more than 10.5 mL of whole blood per kilogram of body weight, including samples
2. Donor interval- 8 weeks after WB donation ? whole blood
3. MD must evaluate medications
4. Medications taken within 48 hrs that irreversibly affect platelet function *(i.e., aspirin)* may not be used as the only source for platelets but can be part of a platelet pool

Criteria for Allogeneic Donor Selection

• Age	≥ 16 yrs. or conform to state law no max (evaluate by M.D.)
• Temperature (oral)	≤37.5˚C or ≤ 99.5˚F
• Blood Pressure	must be "within normal limits"; no longer defined by AABB as 180/100; institution defined.
• Hgb/ Hct	≥12.5g%/ ≥ 38%
• Weight	minimum 110 lb / 50 kg

hemoglobin

hematocrit

American Association of Blood Banks

REMEMBER!

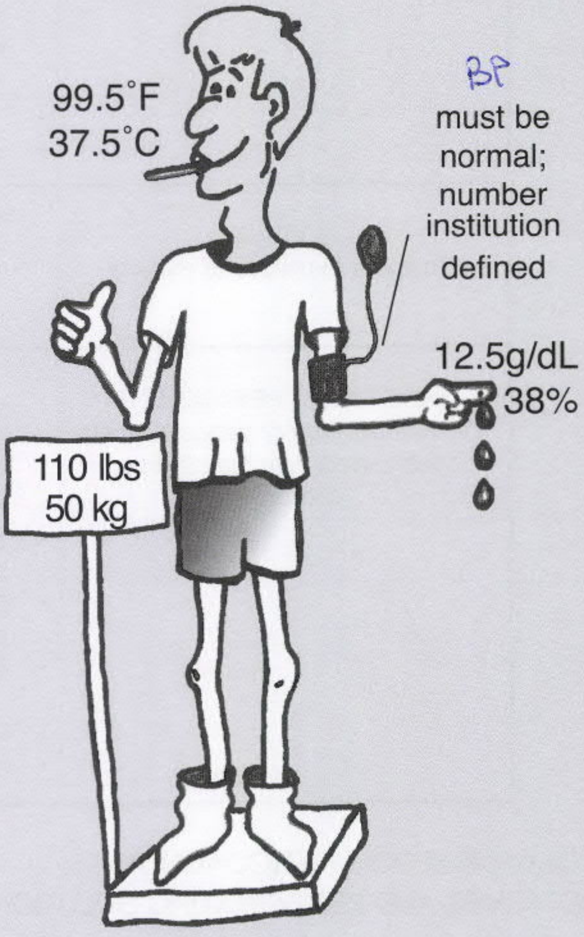
Blood Pressure

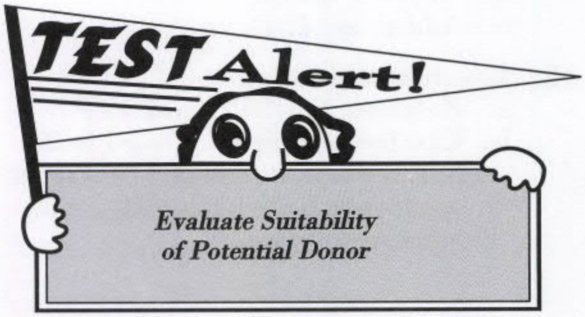
Systolic/ Diastolic

Sky is above Dirt

REMEMBER!

Danny Donor


99.5˚F
37.5˚C
BP
must be normal; number institution defined
12.5g/dL
38%
110 lbs
50 kg



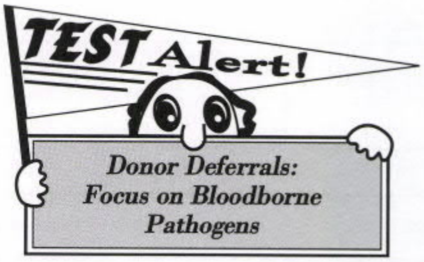
Evaluate Suitability of Potential Donor

Donor Deferral

1 YEAR: Possible exposure to hepatitis, HIV or malaria;	• Hepatitis B Immune Globulin • Possible exposure to hepatitis, HIV & malaria • Recipient of blood/blood products • Tattoo - unless at state regulated facility • Is living with or having sexual contact with a person positive for HBsAg or HBV NAT; is symptomatic for hepatitis C of any other viral hepatitis • Mucous membrane exposure to blood • Skin penetration with instruments contaminated with blood/body fluid • Sexual contact with individual symptomatic for any viral hepatitis, confirmed + for HBsAg / HIV or in high risk category • From completion of therapy for syphilis or gonorrhea or reactive STS • Traveled to endemic area for malaria • > 72 hours in a correctional institution
3 YEAR: Possible exposure to malaria	• Asymptomatic during the time - Visitor/immigrant from area endemic for malaria - Previously diagnosed with malaria
INDEFINITE/ PERMANENT Definite disease or habits strongly associated with bloodborne pathogens	**VIRAL DISEASES:** • Viral hepatitis after age 11 • Confirmed positive test for HBsAg or positive HBV NAT result • Repeatedly reactive test for anti-HBc or anti-HTLV • Donated only unit to recipient who developed post transfusion hepatitis, HIV, or HTLV • Present/Past infection of HCV, HTLV, HIV or *T. cruzi* • Evidence of parenteral drug use **OTHER DISEASES:** • Family history of CJD or risk of vCJD • History of babesiosis

COMMON BLOOD ANTICOAGULANTS, ADDITIVES AND REJUVENATING SOLUTIONS

1. Expiration with anticogulants and additives
   a. ACD/CPD/CPD2 - 21 days
   b. CPDA-1 -35 days
   c. Additives- 42 Days
2. Rejuvenating solutions
   a. Restores 2, 3-DPG and ATP
   b. Can freeze unit or, if used in 24 hrs., can be stored at 1-6C *(must wash cells before transfusion to remove solution)*


TEST Alert!
Donor Deferrals: Focus on Bloodborne Pathogens

TEST Alert!
Knowledge of Anticoagulants/Rejuvenating Solutions & Expiration Times

AUTOLOGOUS DONATIONS

1. Donations for self; no age limit
2. Hct ≥ 33%; Hgb ≥ 11 g/dL
3. No bacteremia
4. Collection > 72 hours prior to surgery or transfusion
5. Autologous units — segregate from allogeneic units, only used for original donor
6. Low volume collections
   a. Use regular blood bags; volume drawn <10.5 mL/kg body weight for minimum weight (*450 + 45 mL plus testing samples*)
   b. If 300-404 mL drawn, label as Red Blood Cells Low Volume (*components may not be made from these units*)

HEMAPHERESIS/APHERESIS COLLECTION

1. Donor criteria same as for whole blood
2. Limits number of donor exposures
3. Apheresis instruments can selectively remove needed component and return components not needed
4. Cytapheresis
   a. Platelets, granulocytes and leukocytes
      ❖ donations at least 2 days apart and no more than 2 in any 7 day period
      ❖ if RBC cannot be returned, must wait 8 weeks
   b. RBCs
      ❖ deferral is 16 weeks for 2 unit RBC apheresis
      ❖ a two unit RBC donation must not decrease donors hematocrit below 30% or hemoglobin below 10g/dL

Appropriate Use of Autologous Units

Terms Associated with Apheresis

	SEPARATION & COLLECTION OF
Cytapheresis	cells
Plasmapheresis	plasma
Plateletpheresis	platelets
Leuka/Granulocytapheresis	leukocytes/granulocytes

   c. Hematopoetic progenitor cells
      ❖ Can be collected from peripheral circulation

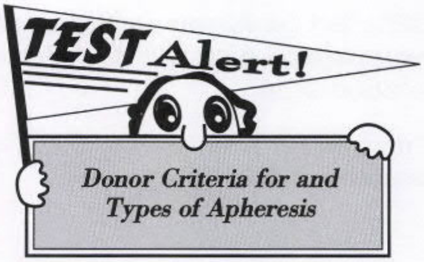
5. Therapeutic
   a. removal of a blood component, e.g., platelets, leukocytes, RBCs
   b. removal of blood substance, e.g., protein, immune complexes in plasma and high molecular weight particles

HEMATOPOIETIC PROGENITOR AND STEM CELLS

1. Used to reconstitute bone marrow post chemotherapy/irradiation or to replace abnormal marrow cells with normal marrow cells *(congenital immune deficiencies, anemias, malignant disorders of bone marrow, red cell disorders, etc.)*
2. Cells obtained from bone marrow, umbilical cord blood and peripheral blood *(apheresis)*
3. Allogeneic marrow — HLA-identical match lowers risk of GVHD; ABO compatibility not required

Human leukocyte antigen, the (MHC) major Histocompatibility complex in human

Graft versus host disease



Donor Criteria for and Types of Apheresis

Donor Blood Processing

TESTS PERFORMED ON DONOR BLOOD

ABO	Resolve discrepancies
Rh	Weak D determination on D negatives
Antibody Screens	Clinically significant antibodies; FFP (Fresh frozen plasma) cannot be prepared from these units; platelets and cryoprecipitate can (*contain minimum plasma volume*)
Serologic tests	Examples: RPR (rapid plasma reagin) for syphillis antibody to *T. cruzi*
Viral Diseases	HBsAg — Hepatitis B surface antigen Anti-HBc — Hepatitis B core antigen Anti- HCV Anti- HIV 1/2 Anti- HTLV I/II — Human T-cell lymphotropic virus NAT (Nucleic Acid Testing) Testing for - HIV 1 - RNA - HCV RNA - West Nile RNA - HBV DNA

Components/Transfusion Practice

WHOLE BLOOD

1. Used in cases of severe shock *(blood loss ≥ 25% blood volume)* needing rbc's for oxygen and plasma for volume
2. Rarely used (due to increased use and availability of components) except for autologus transfusions

RED BLOOD CELLS (PACKED CELLS)

1. Red cells with plasma removed
2. Provides same oxygen carrying capacity as whole blood with less volume
3. <80% hct *(indicates sufficient plasma removal)*; 55-65% hct if additive solution used
4. 1 unit raises hemoglobin *(hgb)* 1g or hematocrit *(hct)* 3%

5. Changes in plasma during storage *(1-6C)*

NH_4 ↑ *pH* ↓
K+ ↑ *Na+* ↓

6. Unit of blood cannot be returned and reissued if >10°C or if seal disturbed

WASHED RED CELL

1. Plasma removed by successive saline washes *(automated instrument)*
2. Primarily used to prevent allergic response to plasma proteins and anaphylactic shock in IgA deficient patients with anti-IgA *(IgA is in normal plasma)*; removes anti-HPA-1a from maternal blood used to neonatal transfusions; removes complement
3. Expires 24 hours after seal of original unit broken

APHERESIS RED BLOOD CELLS

1. Hemoglobin should be >60g in individual units or >50g in 95% of units tested
2. Leukoreduced - ≤ $5x10^6$ leukocytes / unit with final hgb of ≥ 51g in individual units or 42.5 g in 95% of units tested

LEUKOCYTE REDUCED RED CELLS

1. 85% of red cells retained
2. Final wbc count < 5×10^6 to prevent febrile nonhemolytic reactions, HLA alloimmunization, and the transmission of CMV
3. Preparaton by filtration
4. Used primarily for patients with repeated febrile nonhemolytic *(FNH)* reactions; usually due to presence of cytokines released from white cells or alloimmunization to HLA or leukocyte antigens

FROZEN CELLS/ DEGLYCEROLYZED CELLS

1. Cells protected from ultra low temperatures by cryoprotective agent *(40% glycerol)*
2. Used for storage of autologous units and "rare" units; expires in 10 years

3. Must be thawed at 37C and glycerol removed prior to transfusion
4. Stored at $\leq$-65C; 1-6C for 24 hours after deglycerolizing (*open system*)

FRESH FROZEN PLASMA (FFP)

1. Prepared by separating cells and plasma by centrifugation and freezing plasma within 8 hours of collection
2. Expires 1 year from date of collection when stored at $\leq$ -18C or 7 years stored at $\leq$ -65C
3. Once thawed (*between 30-37C*), expires in 24 hours, if stored at 1-6C
4. Must be ABO compatible with recipient cells; not necessarily ABO identical
5. Used for multiple coagulation deficiencies, Factor XI deficiency, and other congenital deficiencies for which no concentrate is available
6. Collection is from males or never-pregnant females to prevent TRALI

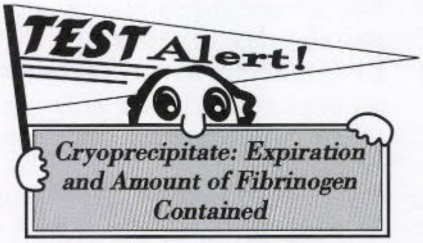
TEST Alert!
FFP:
Determine compatibility and storage requirements

PLASMA FROZEN WITHIN 24 HOURS OF PHLEBOTOMY (PF24)

1. Plasma frozen to -18° C within 24 hours of collection from whole blood or apheresis
2. Kept at 1-6°C for 24 hours and then frozen at $\leq$-18° C

CRYOPRECIPITATE (CRYOPRECIPITATED ANTIHEMOPHILIC FACTOR)

1. When FFP frozen within 8 hours of whole blood collection is thawed at 1-6C, a cold insoluble portion of plasma forms — CRYO
2. CRYO is separated from thawed FFP and refrozen within one hour
3. Must contain $\geq$150 mg of fibrinogen and $\geq$80 IU/bag of Factor VIII
4. Also contains vWF, ristocetin cofactor activity, Factor XIII and fibronectin
5. Store at -18°C for 1 year from date of phlebotomy; Room temperature after thawing
6. Transfuse within 6 hours of thawing; 4 hours after pooling in an open system; 6 hours after pooling in a closed system
7. Most commonly used to replace fibrinogen loss due to DIC and/or massive bleeding or for dysfibrinogenemia with active bleeding


TEST Alert!
Cryoprecipitate: Expiration and Amount of Fibrinogen Contained

FACTOR CONCENTRATES / RECOMBINANT PRODUCTS

1. Recombinant *(most common*) or virally inactivated Factor VII concentrate- treat moderate to severe Hemophilia A and von Willebrand disease (*use Factor VIII labeled as containing vWF*)
2. Prothrombin complex concentrates (*virally inactivated*), Recombinant, or virally inactivated Factor IX concentrate- treat Hemophilia B
   a. Prothrombin complex concentrates contain vitamin-K dependent factors: II, VII, IX, and X; may increase risk of thrombosis
3. Recombinant activated Factor VIIa- treat Hemophilia A and B in patients with inhibitor antibodies (*bypass Factor VIII in cascade*) or those with Factor VII deficiency
4. DDAVP- used for mild hemophilia A and type 1 vWD; increases circulating Factor VIII and vWF.

TEST Alert!
Identify Factor Deficiencies and Select Appropriate Component for Transfusion

PLATELETS/ PLATELETPHERESIS

1. Prepared from whole blood *(stored at 20-24C prior to processing)* or apheresis; whole blood processing listed below
    a. Light spin *(to remove red cells)* followed by heavy centrifugation (*to spin down platelets and white cells*)
    b. Express supernatant plasma into another bag for freezing (*FFP*)
    c. Remaining plasma, platelets and white cells = platelets

2. Conditions
    a. For severe thrombocytopenia and platelet dysfunction
    b. Prophylactic use of platelets when platelet count is low is controversial *(threshold depends on patient's risk of bleeding)*
    c. Contraindicated in TTP and heparin-induced thrombocytopenia (*HIT*)

3. Platelets from donors who are within 48 hours of taking drugs *(e.g., aspirin)* that impair platelet function should not be used as a "single source" *(apheresis product or single unit for a newborn)*

4. Platelet refractoriness- the lack of expected response is usually due to antibodies to HLA class I antigens or platelet specific antigens

5. Transfusion - in average sized adult
    a. 1 unit of platelets raises platelet count 5,000 – 10,000/uL
    b. 1 apheresis unit raises the platelet count 20,000- 60,000/uL
    c. Transfuse within 4 hours after pooling in an open system

6. pH $\geq$6.2 at end of storage; stored in volume of plasma necessary to maintain pH, usually 40 – 70 cc for whole blood derived platelets

7. $\geq 5.5 \times 10^{10}$ platelets/unit in 75% of units tested or $\geq 3 \times 10^{11}$ platelets/plateletpheresis in 90% of units tested

8. Whole blood derived leukoreduced platelets - $<8.3 \times 10^{5}$ leukocytes; leukoreduced pooled platelets or plateletpheresis product - $<5 \times 10^{6}$ leukocytes in 95% units tested

9. Store with continuous gentle agitation at 20 – 24C *(room temp)*

10. Outdate is 5 days

11. Must have method to detect and limit bacterial contamination

12. May have some residual RBCs; consider administering RhIg to D neg women of childbearing age who have received D pos platelets

GRANULOCYTE PHERESIS

1. Obtained by apheresis

2. Granulocyte colony-stimulating factor (G-CSF) increases yield

3. Used for neutropenic patients with documented gram negative sepsis who have not responded to antibiotics

4. Can transmit CMV, induce HLA immunization, and cause GVHD, if not irradiated

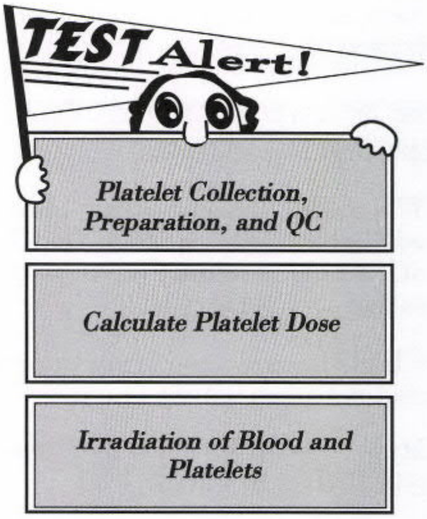
5. Stored without agitation at 20 – 24 C for up to 24 hours, but should be transfused ASAP

6. Should be ABO-compatible with recipient; crossmatch if >2mL RBCs

IRRADIATED BLOOD AND COMPONENTS

1. Prevents graft (*donor lymphs*) vs. host disease (*GVHD*) *(inactivates T cells)*

2. For anyone at GVHD risk: fetus receiving intrauterine transfusion; donor is blood relative of recipient; donor is HLA matched; or congenital immunodeficiency



Platelet Collection, Preparation, and QC

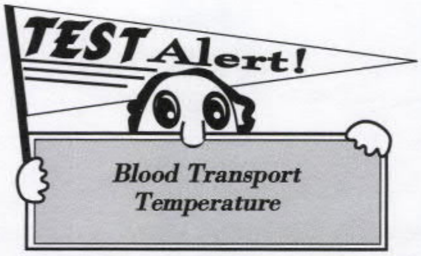
Calculate Platelet Dose

Irradiation of Blood and Platelets

3. **Minimum of 25 Gy (*gray*) or 2500 cGy *(centigray)***
4. **RBCs expired on original outdate or 28 days after irradiation, whichever is first**

MISCELLANEOUS

1. **Transporting blood and components**
   a. **Red cells kept at 1 - 10C**
   b. **Platelets and granulocytes kept at 20-24C**
   c. **Frozen components kept frozen**
2. **Expiration of blood/components when seal is broken *(packing cells or pooling components):***
   a. **Products stored at 1-6C = 24 hrs**
   b. **Products stored at 20-24C = 4 hours**
3. **Pooling components:**
   a. **If red cells visible in pooled product, patient plasma antibodies should be compatible with those red cells**
   b. **Expiration of pooled components**
      - ❖ ***Platelets - 4 hours (open system)***
      - ❖ ***Cryoprecipitate - 4 hours (open system); 6 hours (closed system)***



Blood Transport Temperature

Expiration Times for Various Components and Situations

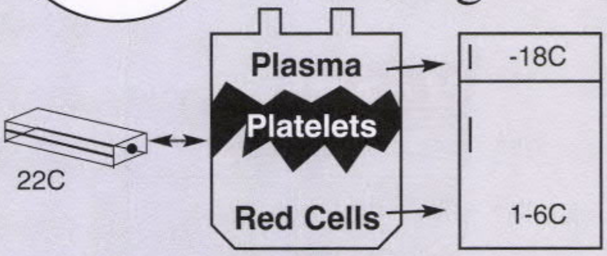
Storage & QC of Blood / Blood Components

Component Quality Control

COMPONENT	STANDARDS	STORAGE
Red Blood Cells	Hct 80% *(Maximum)*	1-6C (Closed system)21 days (ACD, CPD, CP2D), 35 Days (CPDA-1), 42 days (Additive); 1-6C (Open System), 24 hours
Leukocyte Reduced Cells	< 5 X 10^6 leukocytes in 95% units tested	1-6°C, Same as Red Cells
Apheresis RBCs	> 60g Hgb (*individual units*); must have >50g hgb in 95% units tested	1-6^{0}C; CPDA-1 – 35 days; additive system – 42 days; open system 24 hours
Apheresis RBCs- Leukocyte Reduced	>51g hgb (*individual units)* or >42.5 g hgb in 95% units tested	1-6^{0}C; CPDA-1 – 35 days; additive system – 42 days; open system 24 hours
Frozen Red Cells	$\geq$80% of Original Red Cells, Adequate Removal of Cryoprotective Agent (*glycerol*)	10 Years, -65°C or Colder (*40% Glycerol); -120°C (20% Glycerol*); 24 Hours Once Deglycerolized
Fresh Frozen Plasma	Frozen to $\leq$ –18°C or $\leq$ –65°C within 8 hours	12 months, $\leq$ –18°C 7 Years $\leq$ -65°C or Colder
Cryoprecipitated AHF	$\geq$ 80 IU/Unit; 150mg Fibrinogen	12 months, $\leq$ –18°C
Platelets, Single Donor *(Closed System)*	$\geq$ 5.5 x 10^{10} plt/unit in 90% of Units Tested; pH 6.2 or Greater in 90% Units Tested at Maximum Storage Time	5 Days, 20-24°C with Constant Agitation
Platelets, Pooled (*Open System)*	Same as Closed System	4 Hours, 20-24°C with Agitation
Platelets, Leukocyte-Reduced	$\geq$ 5.5 x 10^{10} Platelets in 75% of Units Tested; < 8.3 x 10^5 Leukocytes in 95% of Units Tested; < 5 x 10^6 in Pooled Platelets	Same as Platelets
Apheresis Platelets/ leukoreduced	$\geq$ 3.0 x 10^{11} plt/unit in 90% of Units Tested pH 6.2 or Greater at Maximum Storage Time, <5x10^6 leukocytes in 95% units tested	5 Days, 20-24°C with Constant Agitation 24 Hours *(Open System)*
Apheresis Granulocytes	$\geq$ 1.0 x 10^{10} Granulocytes in 75% of Units Tested	24 Hours, 20-24°C

REMEMBER!

Components Storage



To help you remember storage temperatures for the basic components: Think of a unit of whole blood sitting by a refrigerator. The **plasma** goes straight across to the freezer and is stored at **-18C** or colder. The **red cells** go across into the bottom of the refrigerator and are stored at **1-6C**. Like plates in your home are stored on a shelf at room temperature, **platelets** are stored on a rotating shelf at **20-24C**.

Component Therapy

What Would You Do If . . .

1. An O negative patient needs 6 units of platelets. Only O positive platelets are available. What needs to be considered in this case?

Ans. **The platelets once pooled have an expiration of 4 hours. Since the patient is O negative, the physician may want to consider RhIG. This will depend on the patient *(Female of childbearing age? Elderly? Diagnosis? Probability of getting more platelets, etc.)***

2. A unit of red cells was issued to the OR at 2:00 a.m. It was returned at 2:45 am unentered. It was kept at 2C in a blood bank monitored cooler. Can this unit be accepted for reissue to another patient?

Ans. **Yes, a unit of RBCs can be reissued if it was not entered and was maintained between 1 - 10C during storage and transportation. There should be at least 1 segment still attached to the donor bag.**

3. A patient with a mild case of vonWillebrand's disease suffered minor cuts and bruises in a car accident. What should the physician consider as the treatment of choice in this situation?

Ans. **Patients with mild vonWillebrand-disease can usually be treated with DDAVP. This drug causes release of vWF from endothelial cells causing the plasma level of vWF to increase. More serious cases usually require Factor VIII concentrates (*some are available with vWF*) or cryoprecipitate.**

4. What is the component of choice for a patient in DIC with a low fibrinogen level?

Ans. **Cryoprecipitate is used because of the high concentration of fibrinogen. FFP can be used to restore the depleted coagulation factors, but the volume needed to restore fibrinogen is usually too large.**

5. A patient was diagnosed as Hemophilia A with inhibitors. Why would transfusion of recombinant Factor VIIa be better than transfusion of Factor VIII?

Ans. **The Factor VIIa would bypass the Factor VIII and activate Factor X in the coagulation cascade to achieve hemostasis. If Factor VIII were used, it would be neutralized by the inhibitors (antibodies to Factor VIII) and the patient would still be bleeding.**

6. What is the correct pH that must be maintained for platelets through the end of storage?

Ans. **> 6.2**

7. What is the maximum number of leukocytes that are allowed in a Red Cell Unit for the unit to be considered Leukoreduced?

Ans. **5×10^6 leukocytes/unit**

8. Why and when should Rhlg be considered when transfusing platelets?

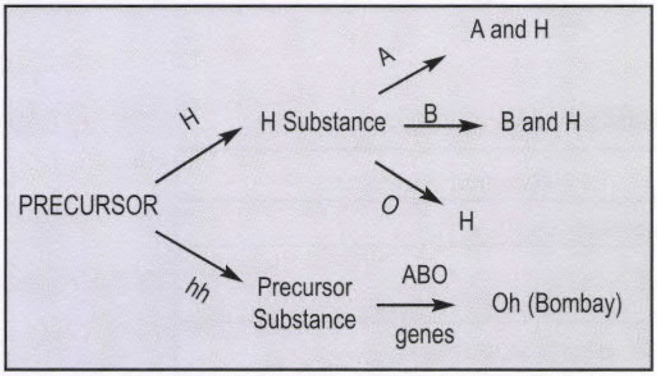
Ans. **Red cells that remain in the unit may carry the D antigen (platelets do not). If the apheresis donor is D positive and the recipient is D negative or if there are some D positive donors in the pool of platelets, the residual RBCs can stimulate an immune response. If the recipient is a female and of childbearing age, it may be prudent to provide RhIg in order to prevent alloimmunization.**

ABO System

ANTIGENS OF ABO SYSTEM

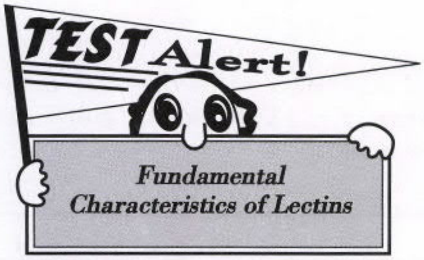
1. ABO antigens defined by immunodominant sugar on cell surface

2. Genetic pathway



3. Subgroups of A
   a. A_1 and A_2
      ❖ *Principal subgroups of A*
      ❖ *Serological difference based on reactivity with anti-A_1 (Dolichos biflorus* or human anti-A_1). (*lectin - plant or seed extract diluted to agglutinate specific human blood group antigens)*
         ☞ A_1 cells are agglutinated
         ☞ A_2 cells are NOT agglutinated

CELL	ANTI-A1
A_1	pos
A_2	neg
A_3	neg



   b. Other subgroups (*A3, Ax, etc*) contain less A antigen and more H antigen

RELATIONSHIP OF ABO, H, SE, AND LE

1. Lack of H is genetically hh *(Bombay phenotype)*
   a. H (HH orHh) is needed for the attachment of A and/or B sugars; H is converted into A and/or B
   b. Group O has the greatest amount of H and A1B has the least amount of H.
      O>A2>B>A1>A1B
   c. Bombays who are hh will have no A or B antigens and the type as group O; O_h phenotype
   d. Anti-H lectin (*Ulex europaeus)* will NOT agglutinate Bombay cells *(hh)* but will agglutinate O cells *(HH or Hh)*

2. Se *(secretor)* gene allows expression of A, B, H, and Le^b in body fluids

3. Le antigens are plasma antigens which adsorb onto red cells as individual matures
   a. Individual with H and Le (*but not Se gene*) genes will have H and Le^a on red cells and Le^a only in saliva *(Se not needed for presence of Le^a in saliva, but it is for H)*
   b. Individual who has H, Se and Le genes will have H and Leb on the red cells and H, Leb, and decreased amounts of Lea in the body fluids

CELL TYPING (FORWARD/ FRONT TYPING) BY TUBE METHOD see page 20 for gel and solid phase testing

1. Reagent anti-A and -B are designed so testing is performed at room temperature
2. Unknown cells + antisera = NO agglutination *(cells lack antigen to which antisera [reagent antibody] corresponds)*
3. Unknown cells + antisera = agglutination *(cells possess antigen to which antisera corresponds)*

REAGENT ANTI-A	REAGENT ANTI-B	INTERPRETATION
+	0	Group A
0	+	Group B
+	+	Group AB
0	0	Group O

SERUM TYPING (*REVERSE TYPING*)

1. **Performed at room temperature with saline suspended known group A_1 and B red cells; optimum reactivity of serum anti-A and anti-B is 4C**
   a. **Unknown serum + reagent red cells = NO agglutination *(serum lacks antibody to antigen on red cell)***
   b. **Unknown serum + reagent red cells = agglutination *(serum has antibody to antigen on red cells)***

A1 CELL	B CELL	ANTIBODY IN SERUM	INTERPRETATION
0	+	Anti-B	Group A
+	0	Anti-A	Group B
0	0	None	Group AB
+	+	Both	Group O

Discrepancies in ABO Grouping

Problems with Red Cells:	Resolution Techniques
• Rouleaux — Failure to Wash Cells	Repeat with Saline Washed Cells
• Mixture of Cell Types — Example: A or B Transfused with O Cells	Check Transfusion History
• Subgroups (*Example: A_2 with or without anti-A_1*)	Test with Anti-A_1 for A Subgroups
• Unusual Genotypes (Example: Bombay)	Test with Anti-H for Bombay (*Bombay Lacks H Antigen and Cells will not Agglutinate with Anti-H; Bombay Serum will Agglutinate A_1 & B Cells as well as Group O Screening Cells*)
• Disease Processes — Example: Leukemia or Bacteria (*Acquired B Phenomenon)*	Check Patient Diagnosis; ID Beyond Scope of This Review
Problems with Serum:	
• Rouleaux — Due to Increased Serum Proteins (Example: Waldenstrom's or Multiple Myeloma)	Saline Replacement
• Room Temperature or Cold Reacting Antibody (*H, I, M, N, P1, or Lewis, or anti-A1 in an A2 or A2B Individual)* Reacting with their Corresponding Antigens on Reverse Cells	"Mini" Cold Screen or Panel (*Test at Lower Temperature*)
• Age — Elderly (*Antibody Production has Decreased*) or Newborn (*Antibody Production Has Not Reached Optimum Levels*); Missing Antibodies	Check Patient Age; "Mini" Cold Panel (*May Enhance Serum Anti-A or Anti-B so Interpretation will Agree with Cell Grouping*)
• Compromised Immune System — Example: A/Hypogammaglobulinemia	Check Patient Diagnosis; "Mini" Cold Panel (*See below*)
With any ABO discrepancy, must transfuse group O cells until the discrepancy is resolved	

☞ "Mini" Cold Panel

Principle: Used to (1) Enhance serum anti-A and anti-B reactions when they are expected but are not demonstrable using room temperature readings, and (2) Identify "cold" antibodies reacting with other antigens on A_1 and B reagent red cells.

PATIENT SERUM TESTED WITH:				INTERPRETATION
A/B Cells	O Cells	O Cord Cell *(if available)*	Autocontrol	
+	+	0	+	Anti-I
+ or 0	+	+ or 0	0	Unexpected antibody reacting at colder temperatures (*anti-H, -M, -N, -P_1 and Lewis antibodies*)
+	0	0	0	Anti-A or Anti-B

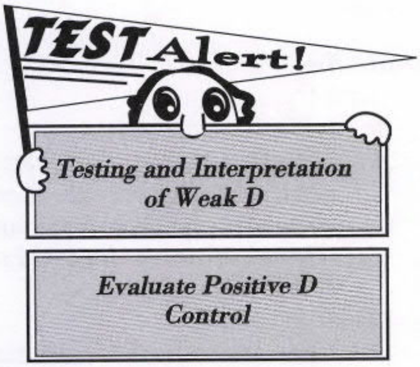
☞ Saline Replacement

Principle: Saline replacement can differentiate rouleaux (*aggregation*) from agglutination. Rouleaux is typically described as having a "stack of coins" appearance when observed with a microscope. When the serum in the test mixture is replaced with saline, the cells dissociate. In assessing rouleaux formation, knowledge of the patient's clinical diagnosis and his/her serum protein content and proportions is helpful. Rouleaux is associated with multiple myeloma and Waldenstrom's macroglobulinemia

Rh System

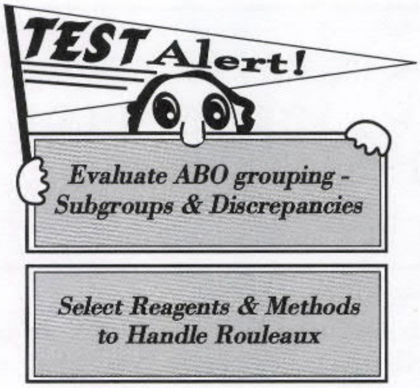
ANTIGENS

		Genes	Antigens
R	D	R^o	Dce
r	no D	R^1	DCe
o or nothing about C or E	ce	R^2	DcE
1 or ′	C	R^Z	DCE
2 or ″	E	r	dce
Z or y	CE	r′	dCe
		r″	dcE
		r^y	dCE


TEST Alert!
Testing and Interpretation of Weak D
Evaluate Positive D Control

D TYPING

1. Most immunogenic of all blood group antigens
2. Test at immediate spin or AHG.
3. Weak D
   a. Negative at immediate spin but positive at AHG. Must have a negative D control for the D testing to be considered positive at AHG.
   b. DONORS MUST be tested through AHG or with sensitive weak D method.
   c. PATIENTS do NOT have to be tested for weak D; consider D negative at immediate spin, can receive D negative blood products.

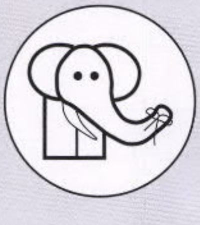

TEST Alert!
Evaluate ABO grouping - Subgroups & Discrepancies
Select Reagents & Methods to Handle Rouleaux

   d. Test all infants of D negative mothers
   e. Record weak-D pos as "D positive"

4. Monoclonal / Polyclonal Anti-D
   a. Separate D control not needed for A, B or O positive cells
   b. A negative reaction with anti-A and/or anti-B in patient is the negative control (*patient cells are not spontaneously agglutinating*)
   c. D control is needed for any AB positive and for any ABO type negative at immediate spin for the D antigen; carry testing through to AHG for weak D typing
   d. If patient is AB positive, use a 6-8% albumin control, autocontrol or DAT (*patient A and B cells typed with reagent anti-A and anti-B will be positive in an AB positive patient, i.e., no negative control*)
   e. Most common cause of a positive D control is a positive DAT

cells	anti-A	anti-B	anti-D	D cont	anti-D AHG	D cont AHG
A	+	0	+			
B	0	+	+			
O	0	0	+			
AB	+	+	+	0		
A	+	0	0	0	0/+	0
B	0	+	0	0	0/+	0
O	0	0	0	0	0/+	0
AB	+	+	0	0	0/+	0

5. High protein anti-D
   a. replaced by monoclonal or monoclonal/polycolonal blend reagents
   b. if used, MUST use D control produced by same manufacturer as the anti-D reagent



REMEMBER!

IgM Antibodies	IgG Antibodies	
anti-I, -H	anti-D	anti-K, -k
anti-M, -N	anti-C, -c	anti-Fya, Fyb
anti-P1	anti-E, -e	anti-Jka, -Jkb
anti-Lea, -Leb	anti-M (some)	

UNUSUAL PHENOTYPES

1. Rh null - no D, C, E, c or e antigens; cells have associated hemolytic anemia since Rh structure is integral part of rbc membrane
2. Deleted cells *(D--)* - missing one or more of normal Rh alleles

RH ANTIBODIES

1. IgG clinically significant
2. May agglutinate at 37C as well as AHG
3. Anti-C, -c, -E, -e react stronger with enzyme-treated cells

Other Blood Group Systems

LEWIS

1. Plasma antigens that adsorb onto RBCs; Le^a and Le^b are not alleles
2. Not on cord cells
3. Antibodies
   a. Do NOT cause HDFN *(Lewis antigens are not on fetal cells and Lewis antibodies usually IgM)*
   b. IgM antibody
      - ❖ *Can be hemolytic*
   c. Usually only seen in Le(a-b-) persons
   d. Often seen in pregnant women who may temporarily become Le(a-b-)

I, i

1. I - absent or weak on cord cells
2. i converts to I as infant matures due to branching of carbohydrate chains; i and I are not alleles
   a. Infants - i positive, I negative
   b. Adults - I positive, i negative
3. Antibodies
   a. Anti-I
      - ❖ *IgM cold antibody*
      - ❖ *Reacts with all adult cells (except rare i adult)*
      - ❖ *May mask clinically significant alloantibody*
      - ❖ *Remove anti-I to detect underlying antibodies by:*
        - ☞ an autoadsorption *(if not recently transfused*) or allogeneic adsorption
        - ☞ RESt adsorption
        - ☞ using IgG AHG instead of polyspecific
        - ☞ prewarming - use with caution; can result in decreased activity of some clinically significant antibodies or cause weak antibodies to be missed

P

1. P_1 antigen strength deteriorates upon storage
2. Antibodies
   a. Anti-P_1
      - ❖ *IgM cold antibody*
      - ❖ *Anti-P_1 (NOT anti-P) can be neutralized to reveal other clinically significant alloantibodies (P_1 substance in hydatid cyst fluid)*
   b. Anti-P
      - ❖ *Autoantibody- IgG; Donath - Landsteiner biphasic antibody found in Paroxysmal Cold Hemoglobinuria*
      - ❖ *Reacts with all P or P_1 positive cells*

MNSs

1. M/N and S/s are both codominant alleles
2. Antibodies
   a. Anti-M and -N
      - ❖ *Usually cold IgM; no HDFN*
      - ❖ *Often show dosage (property whereby antibody reacts strongest with cells having a homozygous*

expression of antigen as opposed to heterozygous cells)
  - ❖ *Will NOT react with enzyme-treated cells (M and N antigens are destroyed by enzymes)*
- b. Anti-M
  - ❖ *Many examples are IgG and can cause HDFN*
  - ❖ *May require acidification of serum to identify*
- c. Anti-S and anti-s - IgG
- d. Anti-U - IgG
  - ❖ *Formed by African American individuals who lack S, s and U*

KELL

1. K and k are codominant alleles
   - a. 91% are K negative
   - b. Antigens inactivated with 2-ME, DTT, or AET

2. Antibodies - IgG

KIDD

1. Jk^a and Jk^b are codominant alleles

2. Antibodies
   - a. IgG
   - b. React STRONGER with enzyme treated cells
   - c. Titers rise and fall rapidly
   - d. Associated with delayed transfusion reactions
   - e. Often show dosage

DUFFY

1. Fy^a and Fy^b are codominant alleles
   - a. Antigens destroyed by enzymes
   - b. 68% African Americans are Fy(a-b-) or FyFy
   - c. Antigen typing – Fy(a+b-)
     - ❖ *Caucasians – homozygous for Fya (FyaFya)*
     - ❖ *African Americans – probably heterozygous for Fya (FyaFy); can cause dosage problems*

2. Antibodies
   - a. IgG
   - b. Weak examples may show dosage. Negative reaction with enzyme treated cells

Relationship Testing (Parentage Testing)

DIRECT AND INDIRECT TESTING

1. RBC blood groups with codominant alleles can be used for parentage testing along with HLA system and DNA analysis

2. Direct exclusion
   - a. marker present in child
   - b. marker absent from father and mother *(most common)*
   - c. father has two different markers in the same system and child lacks either marker *(a group AB father with a group O child)*

Direct Example:

	anti-K	anti-k
Alleged father	+	0
mother	+	0
child	+	+

4. Indirect exclusion
   - a. child lacks a marker that the alleged father must transmit
   - b. father apears to be homozygous for allele child lacks
   - c. father could have silent allele or an allele not tested for

Indirect Example:

	anti-Fya	anti-Fyb
Alleged father	+	0
mother	0	+
child	0	+

Question?

Is the alleged father, tested below, excluded?

Testing:
Father: Fya Fya
Mother: Fyb Fyb
Baby: Fyb Fyb

Answer:

The alleged father appears to be excluded. Must now determine if father is homozygous for Fya or if he carries a silent allele. Father could be FyaFy in which case the baby could be FybFy which would not exclude the alleged father

Evaluate Serologic Reactions Used in Paternity Testing

ABOUT PATERNITY TESTING . . .

- Maternity is Assumed
- In paternity testing, there is a chain of sample custody that must be adhered to in legal cases
- Molecular techniques are replacing serological methods

Antibody Screening & Identification

SCREENING CELLS AND PANELS

1. Commercially prepared group O red cells with a specific distribution of blood group antigens *(screening cells contain 2-3 different cells; panels vary from approximately 10 - 20 different cells)*
2. Screening cells mixed with pt. serum
   a. Serum (*or plasma*)-cell mixture is tested at various temperatures, with different enhancement media, and with antiglobulin reagent *(indirect antiglobulin test [IAT])*
   b. Patient serum (*or plasma)* may also be tested against his own cells *(autocontrol)* to determine the presence of an autoantibody
3. IAT *(Indirect antiglobulin test*)
   a. Antibody attaches to corresponding antigen on red cells at 37C *(may see hemolysis)*
   b. Excess serum/antibody removed by saline washes *(inadequately washing may cause a false negative AHG as residual antibodies and other globulins neutralize the reagent)*
   c. Antiglobulin is added and binds to the antibody on the cells
   d. Positive reaction is indicated by agglutination or ↓ in size of button due to hemolysis at 37C
   e. Check cells *(IgG sensitized cells)* are added to any negative test; should give a positive reaction (*AHG was added and was not neutralized*)
4. Notes
   a. Enzyme treated *(ficin, papain, trypsin and bromelin)* cells are available to compare with panel results of untreated cells
   b. Panel results
      - ❖ *Compare enzyme-treated and untreated cells - enhanced: Kidd, I, P1, Lewis and Rh; destroyed: Duffy, M, N, S and s*
      - ❖ *Dosage - Rh, M, N, Kidd (Jk^a and Jk^b) and Duffy (Fy^a and Fy^b)*
      - ❖ *Strength of reaction may separate multiple antibodies (Panel with 1+ and 3+ reactions may mean two different antibodies)*
      - ❖ *Phase of reactivity (see chart: Antibody Characteristics)*
      - ❖ *Autocontrol - if positive, may indicate a delayed transfusion reaction; if positive along with all panel cells, autoantibody may be indicated*
   c. Prewarmed technique - After proving no clinically significant antibodies also present, eliminate reactions due to cold antibodies
      - ❖ *Warm serum and cells separately to 37°C before mixing together*
      - ❖ *Wash with warm saline prior to further testing (crossmatch, panel, etc.)*
5. Antigen type the patient; patient must be negative for antigen to which the antibody is directed

Enhancement Media

albumin (bovine):
↓ net negative surface charge; only ↑ antibody uptake if under low ionic conditions; Rh antibodies may show at 37°C

Low Ionic Strength Saline (LISS):
↑ antibody uptake which allows ↓ in incubation time

Enzymes (bromelin, ficin, papain, trypsin):
Removes sialic acid to ↓ negative surface charge and promotes cell agglutination, ↑ reactivity of Rh, Kidd and Lewis antibodies; usually ↑ warm and cold autoantibodies; destroys M, N, S, Fya, and Fyb antigens

Polyethylene glycol (PEG):
↑ antibody uptake; removes water which ↑ antibody concentration which promotes antibody uptake

REMEMBER!

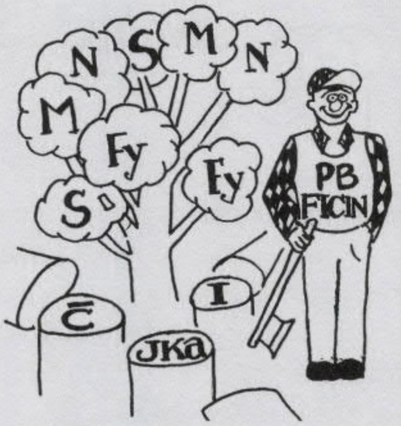
Enzymes:

P.B. Ficin The Lumberjack

P.B. Ficin - The Lumberjack

Papain **B**romelin **F**icin **T**rypsin

M, N, S, s, Duffy antigens on branches are chopped down by enzymes.
Other antigens are exposed (Jk^a, Jk^b, Rh, Lea, Leb, & I).



Antigen Characteristics

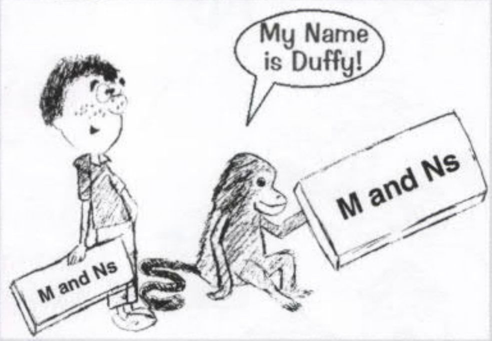
DOSAGE	ENZYMES		CORD CELLS	
	Enhanced	Destroyed	Present	Absent
M, N, S, s Rh Kidd Duffy (weak example of the antibody may show dosage)	Kidd Rh Lewis I P_1	Fya, Fyb M,N,S s (variable when using "in house) treated cells)	i	I Lewis Sda

Antibody Characteristics

Temperature	Media	Antibody Class	Specificity
4-22C	Saline	IgM	Anti-I Anti-H Anti-M Anti-N Anti-P1 Anti-Lea Anti-Leb
37C	LISS/Albumin	IgG	Rh Antibodies: Anti-D Anti-C Anti-c Anti-E Anti-e
		IgM	Cold Antibodies Reacting at a Higher Thermal Range
37C	AHG	IgG	Anti-D Anti-C Anti-c Anti-E Anti-e Anti-Kell Anti-Duffy Anti-Kidd Anti-M (some)
		IgM *(Using Polyspecific)*	Complement Binding Antibodies; Most Common: Anti-I Anti-Lea Anti-Leb

REMEMBER!

Dosage

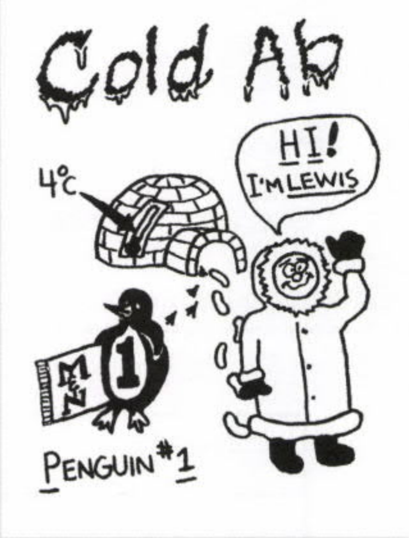


Kid(d)s and **Duffy** the **Monkey** (Rh) eat lots of **M&Ns**

Kidds = anti-Jka, -Jkb
Duffy = anti-Fya, -Fyb
Monkey = Rh antibodies
M&Ns = anti-M, -N, -S, -s
Lots = dosage

REMEMBER!

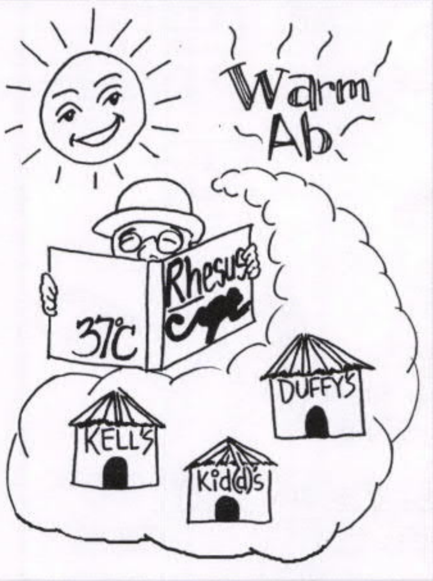
Antibody Temperatures of Reactivity



Let's go on a journey to help you associate blood group antibodies and their temperatures of reactivity:

First, we are going to a place that is very cold. In your mind, see yourself putting on a fur parka, gloves and some very warm boots. Let's get in our kayak and start paddling. We paddle and paddle and just when you think you can't paddle one more stroke, we finally reach land.

Up ahead, we see an igloo and we know that it is very cold here because the thermometer on the igloo reads 4°C. Suddenly out of the igloo comes Lewis the Eskimo. We know he is a very friendly eskimo, because he says, "HI." This tells us the Lewis, H, and I antibodies react in the cold. Next, we see the most amazing sight. Out of that same igloo comes a penguin, the #1 penguin, and under his wing, he is carrying the biggest bag of M&N candy you have ever seen. This tells us the P_1, M & N antibodies also react in the cold. From our journey to the cold, we know that Lewis, H, I, P_1, M and N antibodies best react at 4°C.



To learn about antibodies that react at 37°C, we must travel to a different part of the world. Let's get in our kayak and paddle to jungle territory. We paddle for what seems like days. The sun is shining and it's getting warmer and warmer. We quickly shed our fur parka, gloves and boots. It must be at least 37°C!

Finally, we reach land and gaze at the jungle that lays before us. Up ahead, we see Jungle Jim reading a book about the Rhesus monkeys he sees in the jungle. This tells us Rh antibodies are **seen** at 37°C.

Jungle Jim tells us there are other things in this environment that cannot be seen unless you look very closely. He takes us to the middle of the jungle. We hack our way through vines and branches until we come to a clearing. In the middle of the clearing, we see 2 big huts and a very tiny hut. The big huts have the owners' names on them. One belongs to the Kells, and the other belongs to the Duffys, and the little one must be for their Kid(d)s.

So, from our visit with Jungle Jim, we know the Rh antibodies can be seen directly at 37°C, but to see the Kell, Duffy, Kidd and S, s reactions, we must look further and we do this through antiglobulin testing.

QUICK REVIEW —

Take 3 minutes and repeat the story out loud! Okay, now state the optimum temperature at which the following antibodies react: P_1, Kell, Jk^b, M and Le^a. Congratulations! The story works!

Panel Practice

Here's your chance to show how much you remember about panels. For Panels 1 and 2, state:

1. **What antibody(ies) is (are) present?**
2. **What antibody(ies) is (are) not eliminated?**

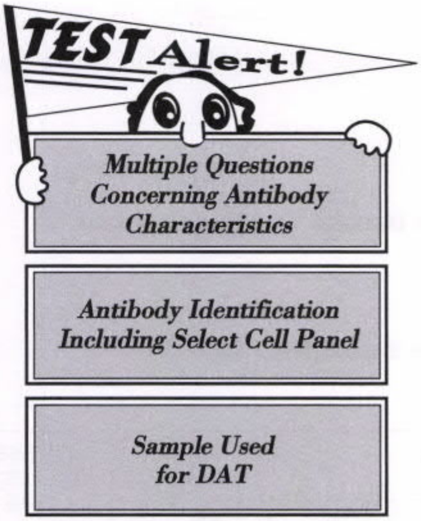
Turn to the end of the chapter for the answers when you are through-p. 32.

Panel 1

Cell No.	D	C	E	c	e	M	N	S	s	P1	Lea	Leb	K	k	Fya	Fyb	Jka	Jkb	Sal IS	LISS 37C 10'	AGH IgG
1	0	+	0	+	+	+	+	+	0	+	+	0	0	+	+	+	+	+	0	1+	2+
2	+	+	0	0	+	0	+	0	+	+	0	+	0	+	+	0	0	+	0	0	0
3	+	+	0	0	+	+	0	+	0	0	+	+	0	+	0	+	+	0	0	0	0
4	+	0	+	+	0	+	+	0	+	+	0	0	+	+	0	0	+	+	0	2+	2+
5	0	0	+	+	+	+	+	0	+	+	+	0	0	+	+	+	+	+	0	1+	2+
6	0	0	0	+	+	+	0	0	+	+	0	+	0	+	+	0	+	+	0	1+	2+
7	0	0	0	+	+	+	+	+	+	+	0	+	+	+	0	+	0	+	0	1+	2+
8	0	0	0	+	+	+	+	0	+	+	+	0	0	+	+	+	+	0	0	1+	2+
9	0	0	0	+	+	+	0	+	0	+	+	0	0	+	+	+	0	+	0	1+	2+
10	0	0	0	+	+	+	+	0	+	0	0	+	0	+	+	0	+	+	0	1+	2+
11	0	+	0	+	+	0	+	+	+	+	0	0	0	+	+	+	+	0	0	1+	2+
AC																			0	0	0

Panel 2

Cell No.																			LISS 37C 10'	AGH IgG	Enzyme	
	D	C	E	c	e	M	N	S	s	P1	Lea	Leb	K	k	Fya	Fyb	Jka	Jkb			37C	AHG IgG
1	0	+	0	+	+	+	+	+	0	+	+	0	0	+	+	+	+	+	0	1+	0	0
2	+	+	0	0	+	0	+	0	+	+	+	0	0	+	+	0	+	+	0	1+	0	0
3	+	+	0	0	+	+	0	+	+	0	+	0	0	+	0	+	+	0	0	0	0	0
4	+	0	+	+	0	0	+	0	+	+	0	0	+	+	0	0	+	+	0	2+	0	2+
5	0	0	+	+	+	+	+	0	+	+	+	0	0	+	+	+	+	+	0	1+	0	0
6	0	0	0	+	+	+	0	0	+	+	0	+	0	+	+	0	+	+	0	1+	0	0
7	0	0	0	+	+	+	+	+	+	+	0	+	+	+	0	+	0	+	0	2+	0	2+
8	0	0	0	+	+	+	+	0	+	+	+	0	0	+	+	+	+	0	0	1+	0	0
9	0	0	0	+	+	+	0	+	0	+	+	0	0	+	+	+	0	+	0	1+	0	0
10	0	0	0	+	+	+	+	0	+	0	0	+	0	+	+	0	+	+	0	1+	0	0
11	0	+	0	+	+	0	+	+	+	+	0	+	0	+	0	+	+	0	0	0	0	0
AC																			0	0	0	0


TEST Alert!
Multiple Questions Concerning Antibody Characteristics
Antibody Identification Including Select Cell Panel
Sample Used for DAT

DAT *(DIRECT ANTIGLOBULIN TEST)*

1. **Antiglobulin added to 3-4 times washed cells**

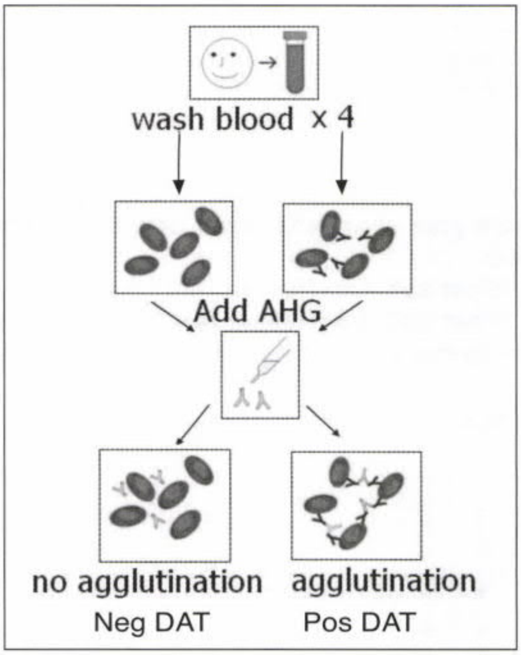
2. **If cells coated in vivo, antiglobulin will react with the IgG antibody and/or complement *(depending on type of AHG used)***

3. **EDTA sample is optimum; EDTA chelates Ca^{++} preventing complement activation by plama antibody *(causes a false positive DAT)***

4. **Add check cells to all negative antiglobulin tests- confirms negatives with IgG coated or complement coated check cells (*proves AHG added and not neutralized due to insufficient washing*)**

Positive DAT

Immune Hemolytic Anemias	
Condition	**Protein Coating Red Cell**
AUTOIMMUNE HEMOLYTIC ANEMIA (AIHA)	
• Warm Autoantibodies (WAIHA)	IgG and/or Complement
• Cold Hemagglutinin Disease (CHD)	Complement
• Mixed Type AIHA	IgG and Complement
• Paroxysmal Cold Hemoglobinuria (PCH)	Complement
DRUG INDUCED HEMOLYTIC ANEMIA (DIHA)	IgG and/or Complement
ALLOIMMUNE HEMOLYTIC ANEMIA	
• Hemolytic Disease of the Newborn	IgG
• Transfusion Reaction	IgG


wash blood x 4
Add AHG
no agglutination
Neg DAT
agglutination
Pos DAT

AHG REAGENTS

1. **Polycolonal- derived from innoculated animals targeting different epitopes**

2. **Monoclonal – derived from hybridoma targeting a single epitope**

3. **Polyspecific- mixture of antibody to IgG and complement components (*usually C3b and C3d*)**

4. **Monospecific- antibody to either IgG or a specific complement component (*usually C3b or C3d*)**

5. **The polyspecific and monspecific can be blended to ensure detection of all epitopes**

TESTS WITH AHG

1. **Perform DAT with polyspecific to screen and monospecific to characterize the globulin as IgG or complement**

2. **Perform IAT with monospecific anti-IgG to avoid cold, complement-binding, clinically insignificant antibodies**

3. **Use IgG coated or complement coated check cells to confirm negative antiglobulin tests; this confirms AHG added and not neutralized (*insufficient washing to removal serum globulins prior to addition of AHG*)**

ELUTIONS

1. Break antigen-antibody bond to release the antibody into solution (*eluate*)

2. Test eluate to determine antibody specificity in cases of positive DAT due to IgG antibody(*ies*), e.g., hemolytic transfusion reactions, HDFN, autoimmune and drug-induced hemolytic anemia

3. Cannot elute off complement

4. Types
    a. No single method best for all antibodies
    b. Lui freeze-thaw and heat - ABO antibodies
    c. Acid or organic solvent methods work best for auto- and allo- warm reacting antibodies

5. Last wash control
    a. Prior to elution, red cells coated with antibody should be thoroughly washed to remove any residual serum (*antibody*)
    b. Test "last wash" (*supernatant*) before performing elution or in parallel with eluate
    c. "Last wash" should show NO reactivity with reagent cells
    d. Positive test results using "last wash" indicate serum antibody contamination of supernatant; if performed before elution, wash again; if performed in parallel, test invalid: repeat

Interpret Eluate Results

Recognize Problems when Testing Eluate Last Wash

NEUTRALIZATION (INHIBITION) TESTS

1. Soluble antigen can bind with antibody to inhibit a reaction with RBCs; allows detection of alloantibodies "masked" by the following antibodies:
    a. Lewis substances – in saliva
    b. P1 substance– in hydatid cyst fluid and pigeon egg whites
    c. Sda substance – most abundant in urine
    d. Chido and Rodgers substances – epitopes of C4 *(complement)*

INACTIVATION

1. Sulfhydryl reagents
    a. AET and DTT – destroys or weakens Kell system antigens
    b. ZZAP: enzyme + DTT – destroys Kell antigens and those antigens destroyed by enzymes (*M,N, S, s, Fya, and Fyb*); can remove immunoglobulins and complement from RBCs to enhance adsorption
    c. DTT and 2-ME – destroys or diminishes activity of IgM antibodies- cleaves disulfide bonds

2. Chloroquine diphosphate
    a. Removes IgG from RBCs (*does not remove complement*)
    b. With IgG removed, cells can be phenotyped
    c. May cause some denaturation of Rh antigens

3. Acid glycine/EDTA
    a. Dissociates antibodies from RBCs
    b. Destroys Kell system antigens

ADSORPTION

1. Used to:
    a. Separate multiple antibodies
    b. Remove autoantibody – reveal alloantibody "masked" by autoantibody
    c. Confirm antigen existence on RBC
    d. Confirm antibody specificity

2. Autoadsorption (*patients own serum and cells*) can be used for patients not recently transfused

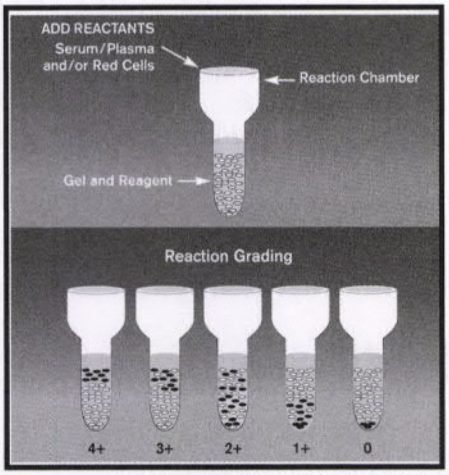
3. Allogeneic adsorptions (*patients serum and other cells*) can be used on patients recently transfused

Additional Technologies to Detect Antigen-Antibody Reactions

COLUMN AGGLUTINATION (*GEL TESTING*)

1. Perform serological work in special chamber with controlled centrifugation
   a. Gel acts as a filter - unagglutinated cells pass through gel; agglutinated cells cannot.
      - *Negative reactions, the cells settle at the bottom;*
      - *Positive reactions, the cells remain on the top or only partially travel through the gel depending on agglutinate size*

   b. Cells are phenotyped when reagent antisera *(e.g., anti-A, -B, -D, etc.)* is in the gel; cells agglutinate on exposure to antibody during centrifugation
   c. Antiserum/serum/plasma and cells are added in the upper chamber; sensitized cells agglutinated by anti-IgG in the gel and cannot pass -IAT
   d. DAT by gel - no washing needed; only RBCs go through gel and sensitized cells agglutinate when exposed to anti-IgG in the gel



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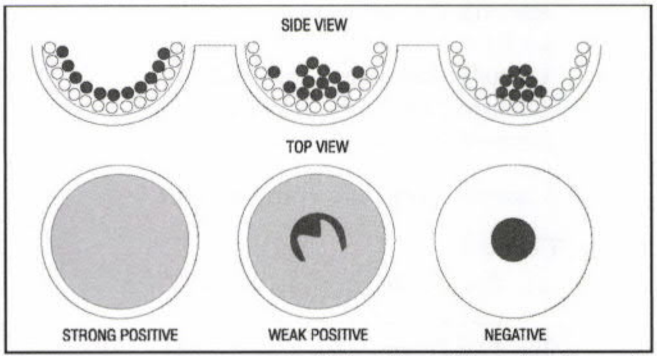
SOLID PHASE

1. Antibody or antigen - fixed to a microwell plate
   a. Antibody fixed to plate
      - *RBCs with the antigen are added*
      - *Antigens adhere to antibody on sides of microplate*

   b. Antigens adhere
      - *Plasma with the antibody is added*
      - *Antibody will adhere to antigen on sides*
      - *Wash; add check cells to attach to antibody*

2. Positive reactions have cells adhering to sides of microwell plate
3. Negative reactions have RBC pellet at bottom of plate since no attachment



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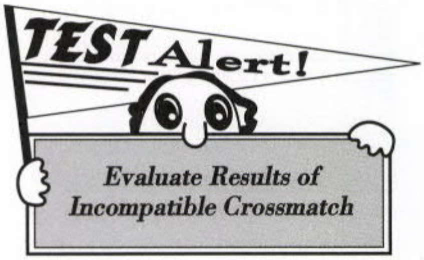
Pretransfusion Testing

1. Sample accompanying request
   a. Serum or plasma from recipient labeled with 2 unique identifiers
   b. Must have system to identify phlebotomist and the date of collection
   c. Retain sample and an RBC segment for 7 days after transfusion
   d. Patients transfused with allogeneic RBCs or pregnant within the last 3 months or transfusion history unknown, a new sample must be drawn within 3 days of transfusion. Day of draw is considered day 0.

2. Tests
   a. ABO group and Rh type
   b. Rh typing; weak D testing confirmation not required for patients
   c. Antibody screens
   d. Crossmatch
   e. Autocontrol not required

3. Compare current results with prior testing
   a. ABO group and Rh type
   b. Any prior difficulties in blood typing



Evaluate Results of Incompatible Crossmatch

c. A list of any clinically significant antibodies
d. Any history of transfusion reactions
e. Any special transfusion requirements

4. Crossmatch
   a. Patient serum mixed with donor RBCs
   b. Observe for agglutination or hemolysis
   c. Demonstrate ABO compatibility
   d. Carry through to 37C incubation with AHG if current antibody screen positive or prior history of clinically significant antibodies

5. Immediate spin or electronic (*computer*) crossmatch if current antibody screen negative and no prior antibody history

   a. Only a test for ABO compatibility is required
   b. Computer only ABO incompatibility check can be used if:
      - ❖ *Computer system is validated*
      - ❖ *Second ABO check on another current sample; checking previous records; or retesting same sample*
      - ❖ *System can verify correct data entry and contains logic to alert if discrepancies occur between donor label and confirmatory tests and between donor unit label and recipient ABO type*

6. Antigen typing
   a. Patients with significant antibodies receive antigen negative units that crossmatch compatible
   b. Probability of finding antigen negative units

      ❖ *Multiply antigen negative* (*compatible*) % converted to decimal

      C = (70% pos; 30% neg); Jka = (75% pos; 25% neg)

      .30 x .25 = .075 or 7.5%*

      * probably find 8 units/100 neg for C & Jka

      ❖ Only need 3 units

      8/100 = 3/X X = 300/8 = 37.5*

      * need to screen ~38 units to find 3 units C negative and Jka negative
   c. Confirm antigen negative status by reacting cells with commercial preparations of the antibody
   d. QC rarely used antisera on day of use
      - ❖ *Positive control: heterozygous cell (e.g., anti-K tested with a Kk cell rather than a KK cell)*
      - ❖ *Negative control: cell without antigen (e.g., anti-K tested with a kk cell)*

TEST Alert!
Select Appropriate Controls for Antigen Typing

When ABO Identical is NOT available.

1. For RBCs
   a. Decide what antibody(*ies*) is (*are*) in the patient's plasma
   b. Transfused cells must lack corresponding antigens
   c. O rbcs can be given to any alternative blood group- A, B or AB
   d. AB can receive A, B and O RBCs
   e. D positive can receive D positive or D negative RBCs
   f. D negative should only receive D negative RBCs. In an emergency, D positive can be administered if no D negative is available. RhIg should follow especially if the recipient is a woman of child bearing age.

2. For Plasma
   a. Decide what antigen(s) is (*are*) on the patient's RBCs
   b. Transfused plasma should lack the corresponding antibody(*ies*)
   c. AB plasma can be given to any alternative blood group - A, B, or O
   d. Group O can receive A, B, or AB plasma

REMEMBER!

For Decisions Involving
- Compatibility Testing Results
- Selection of Units for Transfusion when ABO Identical is NOT Available:

Decide what ABO antibody (ies) is (are) in the patient's plasma. Any red cells LACKING that (those) antigen(s) will be compatible.

REMEMBER! NO WHOLE BLOOD!

		Recipient of RBCs			
Donor		O	A	B	AB
	O	x	x	x	x
	A		x		x
	B			x	x
	AB				x

		Recipient of Plasma			
Donor		O	A	B	AB
	O	x			
	A	x	x		
	B	x		x	
	AB	x	x	x	x

Special Considerations for the Neonatal Crossmatch (< 4 months)

- Initial sample from the infant shall be tested for ABO group (cell type only) and Rh type.
- Test for unexpected antibodies can be done using neonate's or mother's serum or plasma.
- No crossmatch or repeat ABO/Rh tests for neonates under 4 months is necessary during any hospitalization if initial antibody screen is negative using infant or maternal serum or plasma, and group "O", ABO identical, or ABO compatible blood is given and transfused cells are D compatible.

- To issue non-O RBCs, not ABO compatible with maternal ABO, must test for passively acquired maternal ABO antibodies using antiglobulin phase.
- If clinically significant antibody exists, infant must get antigen negative blood or units crossmatch compatible by antiglobulin crossmatch until antibody no longer detected.
- Infants weighing <1200g at birth born to a CMV seronegative mother should only receive blood from a CMV seronegative donor or leukocyte-reduced components.

Adverse Effects of Transfusion

GENERAL

1. Acute or immediate reactions occur within 24 hours of transfusion
2. These include hemolysis, transfusion-related sepsis, TRALI, allergic reactions, TACO, FNHTR.
3. Top three reactions related to transfusion associated mortality are: TRALI, hemolytic transfusion reactions, and TACO.

INTRAVASCULAR HEMOLYTIC TRANSFUSION REACTION- ACUTE

1. Transfused RBCs react with antibodies during transfusion
2. Usually due to clerical error involving ABO system
3. Most common symptom-fever
4. Physiological events
   a. Hemoglobinemia
   b. Hemoglobinuria
   c. Hyperbilirubinemia
   d. Can result in kidney failure and death

EXTRAVASCULAR HEMOLYTIC TRANSFUSION REACTION- DELAYED

1. Usually due to anamnestic response to clinically significant antibodies such as Rh, Kell, Kidd and Duffy; usually occurs after transfusion completed
2. Delayed transfusion reactions
   a. Hours to days after transfusion
   b. Indicated by NO rise or a ↓ in hemoglobin after transfusion
   c. Positive DAT (*key characteristic*)
   d. Often due to Kidd antibodies

TRANSFUSION-RELATED ACUTE LUNG INJURY (*TRALI*)

1. Acute respiratory insufficiency and bilateral pulmonary edema by X-ray without cardiac failure; includes chills, fever, and hypotension
2. Can be life threatening and may be accompanied with transient neutropenia or leukopenia.
3. Caused by antibodies in the DONOR to neutrophils or HLA antigens; occasionally caused by antibodies in recipient
4. Whole blood or plasma from female donors should only be used if she has never been pregnant or has been tested and is negative HLA antibodies

TRANSFUSION ASSOCIATED CIRCULATORY OVERLOAD (*TACO*)

1. Pulmonary edema with hypertension caused by volume overload.
2. Individuals 70 or older are at greatest risk as are individuals who are transfused with large amounts of fluid or modest amount at a high flow rate

FEBRILE NONHEMOLYTIC (*FNH*)

1. Temperature rise of $\geq 1^{\circ}C$ associated with transfusion due to:
   a. Recipient preformed leukocyte antibodies to lymphocytes, granulocytes, or platelets
   b. or infusion of cytokines in donor bag that built up during storage

2. Leukocyte-reduced blood components; pre-storage leukoreduction prevents cytokine buildup

ALLERGIC REACTIONS

1. Recipient preformed IgE antibodies to donor soluble plasma proteins

2. Mild - urticarial - hives with itching
   a. Give antihistamines and continue transfusion

3. Severe - anaphylaxis – systemic symptoms including hypotension, shock, sometimes death
   a. Classic anaphylaxis – IgA deficient patient with anti-IgA reacting with IgA in donor plasma
   b. Give washed cells or plasma components from IgA deficient donors

Transfusion Reactions

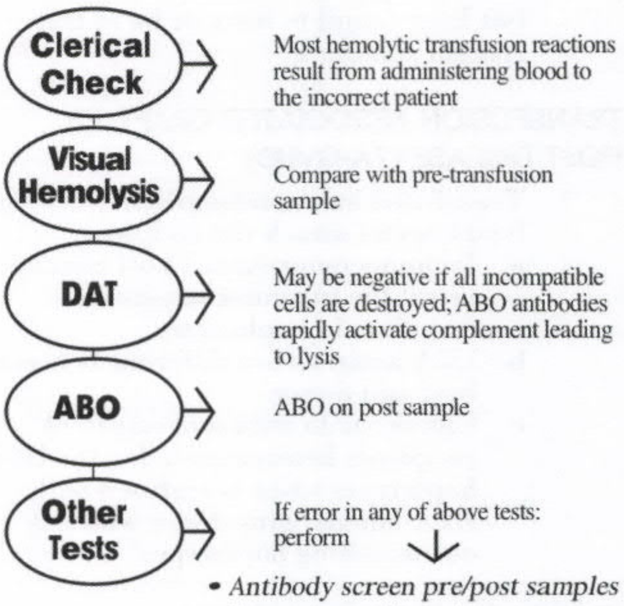
HELP!!
It's too late!!!

TYPE	CAUSE
Hemolytic	Antibody to rbc antigens (usually ABO)
Febrile	Antibody to wbc/plt antigens/cytokines
Urticarial	Antibody to soluble antigen in donor plasma
Anaphylactic	Anti-IgA
Transfusion-Related Acute Lung Injury	Donor antibody to recipient HLA or neutrophil antigens

Keep in Mind:
Positive hemolysis, negative DAT

- Patient in sickle cell crisis
- Thalassemia or G6PD deficient patient
- Unit overheated/frozen
- All cells hemolyzed

Transfusion Reaction Workup



Clerical Check → Most hemolytic transfusion reactions result from administering blood to the incorrect patient

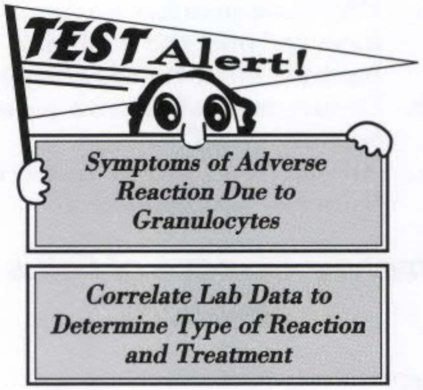
Visual Hemolysis → Compare with pre-transfusion sample

DAT → May be negative if all incompatible cells are destroyed; ABO antibodies rapidly activate complement leading to lysis

ABO → ABO on post sample

Other Tests → If error in any of above tests: perform

- *Antibody screen pre/post samples*
- *Re-crossmatch pre/post samples*



Symptoms of Adverse Reaction Due to Granulocytes

Correlate Lab Data to Determine Type of Reaction and Treatment

TRANSFUSION TRANSMITTED DISEASES

1. Bacterial contamination is now most common since current tests detect most viruses
   a. All platelets must be tested for bacterial contamination before issue

2. Required tests by AABB and/or FDA for transmissible diseases: HBV DNA, HBsAg, anti-HBc, anti-HCV, HCV RNA, anti-HIV-1/2, HIV-1 RNA, anti-HTLV-1/II, WNV RNA, syphilis, and Trypanosoma cruzi

3. Other transmissible disease causing organisms are CMV, Babesia, Malaria, prions (*vCJD*)

4. "Look Back" – Identification of recipients of blood/components from donors who were negative at donation but later found to have or be at high risk for infection

TRANSFUSION ASSOCIATED GRAFT VS HOST DISEASE (TA-GVHD)

1. Transfused immunocompetent lymphocytes attack the recipient
    a. Immunocompromised host cannot reject the immunocompetent transfused lymphocytes
    b. HLA antigens are different between host and donor
    c. Can occur in immunocompetent recipients heterozygous for the HLA haplotypes when transfused with HLA homozygous donor who has one matching haplotype.

TRANSFUSION-RELATED SEPSIS

1. Suspect if high fever with shaking chills and hypotension post transfusion
    a. Diversion pouches on donor bags have reduced the incidence of bacteria entering the component
    b. Occurs most often with platelets because of RT storage
    c. All platelets must be tested for bacterial contamination

Hemolytic Disease of Fetus and Newborn

ETIOLOGY

1. Infant inherits antigen from biological father

2. Mother has corresponding IgG antibody (*sensitized by previous pregnancies or transfusions)*

3. Maternal antibody crosses placenta and binds to fetal cells

4. Coated cells are removed from fetal circulation causing anemia and hyperbilirubinemia

5. Bilirubin has affinity for lipid rich layers of skin and brain and is a potent neurotoxin causing brain damage (*kernicterus*)

6. Phototherapy is treatment of choice; exchange transfusion used when phototherapy is insufficient

INTRAUTERINE TRANSFUSION

1. AABB recommended titer method is saline AHG incubated for 60 minutes at 37°C

2. Critical titer for most antibodies is 16 at AHG; titers can be used to follow the pregnancy; critical titer for anti-K is 8 at AHG

3. Ultrasound and color Doppler ultrasonography can establish severity of HDFN.

4. Purpose and unit selection
    a. Group O, D negative
    b. Negative for antigen to which maternal antibody is directed and crossmatch compatible with maternal plasma
        - ❖ *Should be irradiated to prevent GVHD*
        - ❖ *Should be from CMV seronegative donor or a leukoreduced unit*
        - ❖ *Should be negative for Hgb S*
        - ❖ *Should be less than 7 days old*

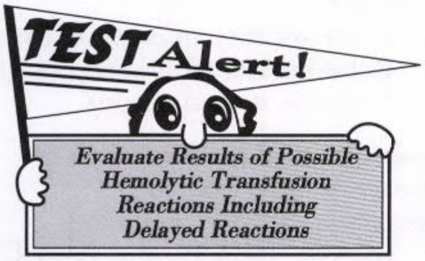
EXCHANGE TRANSFUSION

1. Reduces bilirubin levels and removes maternal antibodies

2. Adds antigen negative cells and removes antibody-coated cells which would ↑ bilirubin levels when destroyed

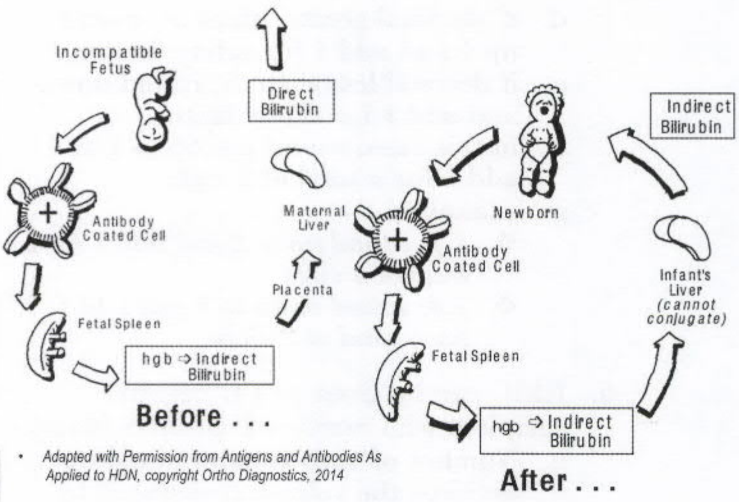
3. Acceptable samples for crossmatch
    a. Maternal sample
    b. Eluate from infant's cells
    c. Infant serum

4. Unit selection
    a. Negative for antigen to which maternal antibody directed *(compatible with maternal antibody)*
    b. Group O, if ABO HDFN; D negative, if Rh HDFN
    c. Age of unit usually less than 5- 7 days old and collected in CPDA-1
    d. Recommend: negative for Hgb S, irradiated, leukoreduced or CMV seronegative


TEST Alert!
Evaluate Results of Possible Hemolytic Transfusion Reactions Including Delayed Reactions

Rh IMMUNE GLOBULIN (*CONCENTRATED ANTI - D*)

1. Antepartum administration given at 28 weeks to all D negative women and given postpartum within 72 hours of delivery to D negative women who deliver D positive infant.
2. Recommended for all D negative women after any abdominal trauma or abortion and any testing such as amniocentesis or cordocentesis.
3. Intramuscular (*IM*) injection or intravenous (*IV*) for the mother - 1 vial (*300 µg or 1500IU*) neutralized 30 mL whole blood (*15 mL RBCs*) fetal-maternal hemorrhage (*FMH*)
4. Must do a test on all RhIG candidates to determine if more than one vial necessary; rosette test usually used for screening
   a. If rosette test is negative, one vial of RhIG should be administered
   b. If rosette test positive, Kleihauer-Betke acid elution or flow cytometry will quantitate fetal maternal bleed


Incompatible Fetus
Direct Bilirubin
Antibody Coated Cell
Fetal Spleen
hgb ⇨ Indirect Bilirubin
Before . . .
Maternal Liver
Placenta
Antibody Coated Cell
Newborn
Indirect Bilirubin
Infant's Liver (cannot conjugate)
Fetal Spleen
hgb ⇨ Indirect Bilirubin
After . . .

- *Adapted with Permission from Antigens and Antibodies As Applied to HDN, copyright Ortho Diagnostics, 2014*

5. Kleihauer-Betke test – fetal cells resist acid elution and appear pink while adult cells are ghost cells
6. Calculation of number of vials with addition of a safety vial
   a. using the Kleihauer-Betke test, count a total of 2000 cells, note the number of fetal and maternal cells
   b. divide the number of fetal cells by 2000 and multiple by 5000 to determine volume of fetal whole blood bleed
      ❖ *8 cells /2000 cells x 5000 mL = 20 mL fetal whole blood bleed*
   c. divide by 30 since 1 vial of RhIg will neutralize 30 mL of fetal bleed
      ❖ *20 mL / 30 mL = .66 vial*

Comparison of HDFN Due to ABO and Anti-D Antibodies

ABO	Rh
↑ Spherocytes	↑ Reticulocytes
DAT weak or negative	DAT positive
Delayed jaundice	Immediate jaundice
Bilirubin rarely > 15 mg%	Bilirubin often > 20 mg%
1st pregnancy: Usually O mother with A baby	Usually not 1st pregnancy: D neg mother with D positive baby

In which type of HDFN would an exchange transfusion be more likely needed? → Rh

Why? → Bilirubin is neurotoxic to the brain and levels > 20 mg/dL *(lower in premies)* can lead to mental retardation and/or death

Laboratory Results

- Cord hemoglobin - decisive factor in deciding whether to perform exchange transfusion
- Bilirubin - most physicians perform exchange transfusion when level approaches 20 mg/dL, lower in premie
- DAT - single most important diagnostic test in diagnosis of HDN after birth
- Occasionally, D group may appear negative at immediate spin due to heavy coating of cord cells with maternal antibody; D and D control will be positive at the antiglobulin phase of testing *(positive DAT)*

   d. if decimal greater than .5, round up 1 and add 1 for safety factor
   e. if decimal less than .5, round down and add 1 for safety factor
   f. in this case, round up .66 to 1 and add 1 for a total of 2 vials
   g. examples:
      - ❖ *1.7; round up to 2 and add 1 for a total of 3 vials*
      - ❖ *1.4; round down to 1 and add 1 for a total of 2 vials*

6. RhIG can be given to a D negative recipient who receives D positive blood.
   a. Number of vials is determined by dividing the volume transfused by 30 for whole blood or 15 for RBCs.
   b. Should be considered for any woman of childbearing age

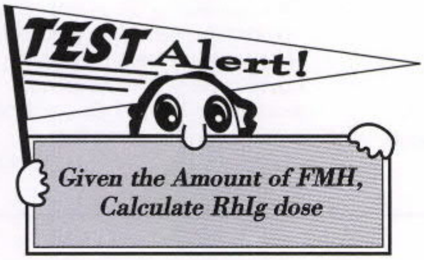
HLA SYSTEM
(HUMAN LEUKOCYTE ANTIGENS)

1. Cell surface glycoproteins
   a. Class I – on platelets and nucleated cells *(mature RBCs may have very small amount)*
   b. Class II – on B lymphs; monocyte/macrophages; activated T lymphs and dendritic cells

2. Contributes to self/non-self recognition; immune responses; coordination of cellular and humoral responses

3. Second in importance only to ABO for long-term survival of transplanted solid organs and most important in hematopoietic progenitor cell transplantation

4. Plays a role in:
   a. Immune-mediated platelet refractoriness
   b. FNH-TR
   c. TRALI
   d. Posttransfusion graft-vs-host disease (*GVHD*)



Given the Amount of FMH, Calculate RhIg dose

5. Used for:
   a. Susceptibility to certain diseases
   b. Relationship (*Parentage*) testing
   c. Forensic investigations

6. Genes located on short arm of chromosome 6 (*major histocompatibility complex*)
   a. HLA-A, HLA-B, and HLA-C – Class I A, B, and C antigens
   b. HLA-DP, HLA-DQ, and HLA-DR – Class II antigens
   c. Inherit haplotypes as autosomal and codominant

7. Detection of HLA antigens and alleles are DNA based or serologic
   a. DNA-based Assays replaced mixed leukocyte culture test
      - ❖ *high sensitivity and specificity*
   b. Serologic – Microlymphocytotoxicity test – HLA-A, -B, -C, -DR, and -DQ.
      - ❖ *Known HLA sera is placed in microdroplet plates*
      - ❖ *Lymphocytes and rabbit complement are added*
      - ❖ *If antibody matches antigens on lymphocytes, complement lyses the cells – lymphocytotoxicity*
      - ❖ *A dye is added and taken up by lysed cells*

8. HLA crossmatch (lymphocyte crossmatch)
   a. Incubate recipient serum with donor lymphocytes from potential donors
   b. Can also be done by Flow Cytometry

9. Solid phase assays
   a. Beads or microparticles are coated with HLA antigens – class I, II, or recombinantly expressed purified HLA antigens
   b. Detect antibody binding using fluorescently labeled AHG

Quality Control

REAGENT QC

1. Reagents – each day of use
    a. Antihuman globulin
    b. Blood grouping reagents
    c. Antibody screening and reverse grouping cells

2. Results - compare with previous results; inactivity implies reagent deterioration

3. Antiglobulin Reagent
    a. Required that IgG sensitized cells be added to all negative antiglobulin tests in antibody detection and compatibility testing
    b. Negative tests imply:
        ❖ *Insufficient removal of serum proteins prior to addition of AHG (insufficient washing which allowed AHG to be neutralized by remaining serum)*
        ❖ *Omission of AHG from procedure*
        ❖ *Must repeat tests*

EQUIPMENT QC

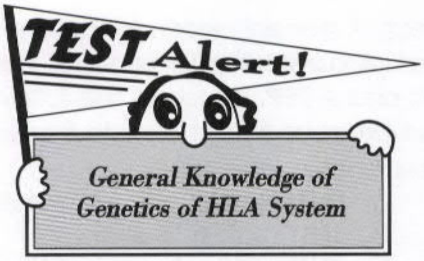
1. Heating blocks/waterbaths: 37C ± 2; observe temperature, day of use

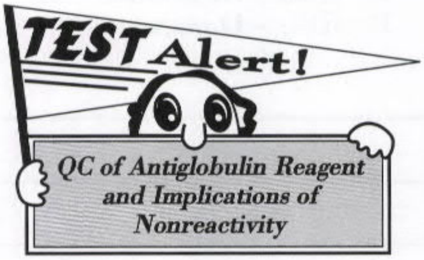
2. Refrigerators: 1-6C daily; temperature charts- weekly; alarm activation checks-quarterly

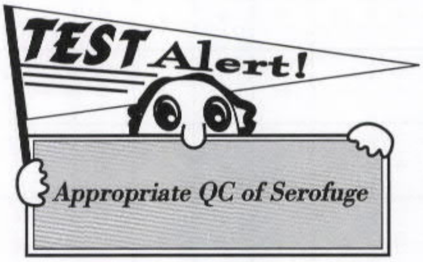
3. Freezers < -18C daily; same as refrigerators

4. Centrifuge/Cell Washer:Speed and timers – quarterly; function – yearly; saline fill volume – weekly

5. Thermometers – tested yearly


TEST Alert!
General Knowledge of Genetics of HLA System


TEST Alert!
QC of Antiglobulin Reagent and Implications of Nonreactivity


TEST Alert!
Appropriate QC of Serofuge

IMMUNOHEMATOLOGY SAMPLE QUESTIONS

1. The following results were obtained when testing a sample from a 20 year old first time donor.

CELL GROUPING		SERUM GROUPING		
anti-A	anti-B	A1 Cell	B Cell	O cell
3+	0	1+	3+	0

The most likely cause of this ABO discrepancy is
   - A. Lack of immune response
   - B. Alloantibody reacting with B cell
   - C. Oh (Bombay)
   - D. Subgroup of A

2. A group A patient needs blood and FFP. The small rural hospital is out of both A blood and A FFP. Which of the following would be your first choice to transfuse to this patient?
   - A. RBC – AB; plasma – AB
   - B. RBC – AB; plasma – O
   - C. RBC – O; plasma – AB
   - D. RBC – O plasma – O

3. For which of the following blood groups is it NOT necessary to run an Rh control if you are using a monoclonal/polycolonal blend anti-D?
   - A. A neg
   - B. A pos
   - C. AB neg
   - D. AB pos

4. The serum in the panel below is from a patient transfused 5 months ago. The most probable antibody is
   - A. Anti-c
   - B. Anti-I
   - C. Anti-k
   - D. Autoantibody

5. Red blood cells which are to be tested with antiglobulin reagent are washed to
   - A. Remove traces of bacterial proteins
   - B. Wash away traces of free hemoglobin
   - C. Remove unbound serum globulin
   - D. Expose additional antigen sites

Cell No.	C	D	E	c	e	K	k	Fya	Fyb	Jka	Jkb	Lea	Leb	M	N	S	s	Sal IS	LISS 37C 10'	AHG (IgG)
1	+	0	0	+	+	0	+	+	0	0	+	0	+	+	+	+	0	0	0	2+
2	+	+	0	0	+	0	+	+	+	+	0	0	+	0	+	0	+	0	0	2+
3	+	+	0	0	+	+	+	0	+	+	0	+	0	0	+	0	+	0	0	2+
4	0	+	+	+	0	0	+	0	+	+	+	0	+	+	0	+	0	0	0	2+
5	0	0	+	+	+	0	+	0	+	0	+	+	0	0	+	0	+	0	0	2+
6	0	0	0	+	+	0	+	0	0	+	0	0	0	0	+	+	0	0	0	2+
7	0	0	0	+	+	0	+	+	+	+	+	0	+	+	0	0	+	0	0	2+
8	0	0	0	+	+	+	+	+	+	+	0	+	0	0	+	0	+	0	0	2+
AC																		0	0	2+

6. An antibody screen gave no reactions at immediate spin or 37C, but showed a 2+ reaction when antiglobulin reagent was added. The most likely antibody causing these results would be anti-
   - **A. I**
   - **B. Jka**
   - **C. Leb**
   - **D. P_1**

7. Which of the following would cause an individual to be rejected as a blood donor?
   - **A. Donor believes he had some form of hepatitis at age 9**
   - **B. Current hemoglobin is13 g/dL**
   - **C. Donor is 65 years old**
   - **D. Donor had chicken pox vaccination 3 weeks ago**

8. Over a two week period, the reactions of your QC antibody show a gradual decrease from 2+ to a very weak positive with your antibody detection cells (*screening cells*). These results most likely indicate
   - **A. Acceptable performance of reagents**
   - **B. Inappropriate antibody specificity**
   - **C. Inconsistent grading of reactions**
   - **D. Deterioration of QC antibody**

9. The following reactions were obtained on testing maternal serum and infant cord cells.

MATERNAL SAMPLE	INFANT CORD CELLS
O negative (D weak test - mixed field)	A Positive
D Control: Negative	DAT: Negative
Antibody Screen: Negative	

   The most likely explanation for these results is a/an
   - **A. ABO grouping error on infant**
   - **B. Detection of antenatal Rh immune globulin**
   - **C. Fetal-maternal hemorrhage**
   - **D. False negative DAT**

10. Based on the following results, select the best conclusion.

    Mother: **MM R^oR^o**
    Alleged Father: **MM rr**
    Child: **MN R^or**

    The alleged father is
   - **A. Not excluded**
   - **B. Excluded by his D antigen**
   - **C. Excluded by his e antigen**
   - **D. Excluded by his M antigen**

11. A 24 year old A negative female was transfused with approximately 65 cc of an A positive RBC unit. How many vials of Rh Immune globulin should this woman receive?
   - **A. 0, she is not an RhIg candidate**
   - **B. 2**
   - **C. 3**
   - **D. 4**
   - **E. 5**

12. The transfusion component of choice for a bleeding patient with a prolonged bleeding time, increased APTT, decreased levels of Factor VIII antigen and impaired aggregation of platelets in response to ristocetin would be
   - **A. Cryoprecipitate**
   - **B. Factor VIII**
   - **C. Fresh frozen plasma**
   - **D. Platelets**

13. How many units of platelet concentrates would be needed to raise the platelet count 150,000/mm3 in an average sized adult?
   - **A. 4**
   - **B. 8**
   - **C. 12**
   - **D. 15**

14. A patient has experienced febrile reactions following 2 red cell transfusions. The best component to use if subsequent transfusions are needed would be
   - **A. Neocytes**
   - **B. Packed red cells**
   - **C. Washed red cells.**
   - **D. Leukocyte-reduced red cells**

15. Cord bloods are washed prior to ABO and Rh grouping to
   - **A. Expose A and B antigens**
   - **B. Remove Wharton's jelly**
   - **C. Eliminate infant's anti-i**
   - **D. Prevent reagent neutralization**

16. Four units of platelets were pooled and issued at 2:00 p.m. At 7:00 p.m. the ward called, said they had never transfused the platelets, and wanted to know if the platelet pool could still be used?

A. No, they outdated at 6:00 p.m.
B. No, the platelets weren't refrigerated on the ward
C. Yes, they won't outdate until 8:00 p.m.
D. Yes, they are good for 24 hours after pooling

17. A group B patient needs blood, but ABO identical blood is unavailable. Which alternative group(s) may be used?

18. A group AB patient needs blood, but ABO identical is unavailable. Which alternative group(s) may be used?

19. A group O patient was crossmatched with group B red cells. Will this incompatibility be detected?

20 A group B patient needs FFP. Which blood group(s) would be acceptable?

21. A group B neg needs 3 units of red cells and 1 unit of FFP. No B neg RBCs or FFP are available. What would be the choice for both components?

22. Four units of O neg RBCs are issued on emergency release to the ER. Immediately after the blood is issued, a blood sample and a request for 4 more units is received. The 4 additional units of O neg RBCs are issued and the type and crossmatch is started. The patient turns out to be an A pos with a negative antibody screen. The crossmatches with the first 8 O neg units are compatible and the antibody screen is negative. The ER calls requesting 6 more units. What ABO/Rh group should these next 6 units be?

ANSWERS AND RATIONALE

1. D

This donor is a probable A2 subgroup with anti-A1. Option A is incorrect because the donor demonstrates anti-A and anti-B in his/her serum. Option B is incorrect because the reaction with the B cells is the expected reaction in an A person. Option C is incorrect because a Bombay forwards as an O. Since the patient serum tested with "O" cells (*antibody screen*) is negative, the antibody has to be reacting with something on the A1 cell that is not on O cells and not reacting with patients own cells – A1 antigen. Must be an anti-A1.

2. C

You must transfuse RBCs that lack the antigens corresponding to patient antibodies and transfuse plasma that lacks antibodies corresponding to the patient antigens. The patient is a group A with anti-B. Therefore, of the answer choices, you could give group O RBCs (*won't react with patient's anti-B*) and AB plasma (*doesn't have anti-A to react with patient RBCs*) Option A, B, and D are incorrect for one or more of the following: patient's anti-B would react with the AB cells and/or the anti-A in the O plasma would react with the patient's RBCs.

3. B

Option A and C are incorrect because an Rh control is needed for any D negative donor in order to perform the weak D test. Answer D is incorrect because a negative reaction with either the anti-A and/or anti-B reagent (*as needed*) shows that the patient cells are not spontaneously agglutinating and acts as the negative control. The AB positive has positive reaction with reagent anti-A and anti-B.

4. D

When serum demonstrates the same strength of reactivity with all cells tested including the autocontrol, an autoantibody is suspected. *(This occurs in patients with warm autoimmune hemolytic anemia.)* Option A is incorrect because cells 2 and 3 lack the c antigen but have reactivity with the serum tested. Option B is incorrect because most examples of anti-I would demonstrate activity at immediate spin/room temperature as opposed to only strong reactions at the antiglobulin phase. Option C is incorrect because the autocontrol is positive and individuals forming anti-k would be k negative. *(The previous transfusion was 5*

months ago and no transfused cells would be in circulation at this time to explain the positive autocontrol.)

5. C

Antiglobulin reagent will react with any serum globulin whether it is in serum or coating red cells. Therefore, if all unbound serum is not removed, it will bind with the antiglobulin reagent and neutralize it. Options A and B do not contain globulin. Option D describes the effect of enzymes on some red cell antigens.

6. B

Most examples of anti-Jka react only at the antiglobulin phase of testing. Options A, C and D are antibodies that generally react at the immediate spin/room temperature phase of testing.

7. D

Options A-C are all ok for donations. Defer for hepatitis after age 11; 12.5 g/dL is lowest for Hgb; there is no age cut off; Option D is incorrect since Chicken Pox vaccination has a 4 week deferral.

8. D

Option A is incorrect because a steady decrease in reaction strength in reagent quality control signifies a loss of antigen or antibody potency. Option B would show consistent readings but at incorrect phases or with incorrect cells *(QC antibody should react with all screening cells at the antiglobulin phase of testing).* Option C would show a more erratic pattern of reactions (*some days stronger, some days weaker*).

9. C.

A positive weak D test in a post partum patient is usually due to a fetal maternal hemorrhage in which the fetal D positive cells have entered the maternal circulation most often at delivery. Option A is incorrect as it is plausible for an O negative mother to have an A positive infant. Option B is incorrect since the maternal antibody screen is negative *(RhIG = anti-D).* Option D is incorrect since hemolytic disease of the newborn is not indicated.

10. D

In relationship (*paternity*) testing maternity is assumed. The child's M antigen is inherited from the mother who appears to be homozygous for M *(MM).* The child's N antigen must be inherited from the biological father. This alleged father does not possess the N antigen and appears to be excluded. Option B is incorrect since the father does not possess a D antigen *(rr=dce/dce).* Option C is incorrect since the child has e as does the father.

11. E

The female is of childbearing age and is an Rh immune globulin candidate. RhIG is expected to counteract 15 cc of RBCs. 65/15 cc = 4.3. Round down to 4 (*round down if the decimal is less than .5*) and add 1 (*safety factor*) for a total of 5. (*Remember, in a fetal-maternal bleed the number of cc is divided by 30.*)

12. A

These laboratory data suggest a patient with Von Willebrand Syndrome. Cryoprecipitate is the only component containing Von Willebrand factor *(vWF)* as well as factor VIII. Both of these are deficient in these patients. It is the vWF that is responsible for the platelet aggregating effect of ristocetin. Since the patient is bleeding, it is more likely a severe case which would warrant cryo. If the case were mild, DDAVP could be used instead. Option B is incorrect because plain Factor VIII does not contain wWF; you would need to use the Factor VIII concentrates known to have vWF. Option C lists FFP as a choice. FFP has cryo in it but has too much volume; you need to give the patient concentrated factors, not volume. Option D is incorrect because you don't need platelets, you need vWF.

13. D

One unit of platelets theoretically increases the platelet count in an average sized adult 5,000 - 10,000/mm^3. 150,000 / 10,000 = 15 units of platelets. The count could actually be as high as 30 units if this patient responds on the lower end: 150,000 / 5,000 – 30 units of platelets

14. D

Most febrile nonhemolytic *(FNH)* transfusion reactions are due to cytokines released from WBCs in the stored blood or to recipient antibodies to antigens on donor lymphocytes, granulocytes and platelets. Patients experiencing a FNH reaction for the first time do not always have a similar reaction with subsequent transfusions. Leukocyte-reduced products are recommended for patients who exhibit 2 or more FNH reactions. Option A and B could both still have WBC and/or cytokines. Option C is a viable option since washing would remove WBCs and cytokines but the question asks for the BEST answer. Washing is too expensive and rarely used for this purpose since the development of leukoreduction filters. Pre-storage leukoreduction works the best since this prevents the buildup of cytokines during storage.

15. B

If cord blood is not collected appropriately, it may be contaminated with Wharton's jelly. This substance, if not removed through saline washes will cause red cells to mechanically "stick" together possibly causing false positive interpretations. Option A is incorrect because the A and B receptors on cord cells are exposed sufficiently for ABO and Rh grouping. Option C is incorrect because infants do not have anti-i. *(Cord cells have i antigen).* Option D is incorrect because Wharton's jelly will not neutralize anti-A, anti-B, or anti-D

16. A

Option B, C, and D are incorrect because none reflect the correct time or storage temperature for pooled platelets. Pooled platelets are good for 4 hours after pooling.

17. Only group O since B individuals have anti-A and O units have no A antigens (*or B antigens!*)

18 Any blood group red cells since AB individuals have no ABO antibodies.

19. Yes, the patient serum contains anti-A, -B and -A,B and will show agglutination when added to the group B cells (*probably a strong reaction at immediate spin*).

20. A group B patient has B antigens on his red cells. NO FFP with anti-B will be acceptable (*therefore no A or O*). Group B (*has anti-A*) or group AB (*no ABO antibodies*) will be fine.

21. O neg red cells is the appropriate ABO group to select. If this is an emergency and no B neg or O neg is available, B pos or O pos would be used. The FFP should be AB since group O and A contain anti-B.

22. The patient should be switched to D positive blood now. The question is whether the A units will be compatible with the passively transfused anti-A and anti-AB after receiving 8 units of O neg RBCs. Additive units contain very little residual plasma, i.e., ABO antibodies. In transfusing a small number of units, there is little risk associated in switching the patient to A pos RBC except for a possible weak transient positive DAT. Even in transfusing 8 units, there may not be a problem unless the patient is a child or small adult. A new post transfusion sample can be requested and tested for ABO antibodies either with an immediate spin crossmatch or a crossmatch carried through to AHG to detect weak ABO antibodies If ABO antibodies are detected, only RBCs lacking the ABO antigen corresponding to the ABO antibody should be used.

Panel 1 answers *(page 18)*

1. Anti-c
2. Anti-E & -K

Panel 2 answers *(page 19)*

1. Anti-Fy^a & Anti-K
2. Anti-E & Anti-Jk^b
   (not ruled out on original panel; eliminated by enzymes)

IMMUNOLOGY AND SEROLOGY

by Patsy Jarreau

TYPES OF IMMUNITY

Active vs. Passive

ACTIVE	PASSIVE
Individual Produces Antibody	Antibody Transferred to Individual
Follows Immunization or Infection	Example: Gamma Globulin Injections, placental transfer
Memory *(lasting)*	No Memory *(temporary)*

What type of immunity is associated with rubella immunization? → Active Immunity

What type of immunity is associated with neonatal (<4 months), syphilitic IgG antibody titers? → Passive Immunity

Natural Immunity vs. Adaptive Immunity

NATURAL *(Innate)*	**ADAPTIVE** *(Acquired)*
Non-Specific	Specific
No Memory	Memory
Examples: Exogenous *(Skin)* Endogenous *(Stomach Acid)* Phagocytosis *(PMNs)* *Natural Killer Cells (NK)*	Examples: T Cells *(cytokines)* B Cells *(antibodies)*

Type of Immunity Associated with Immunization

Type of Cell Producing Antibody

Immunity Associated with Viral Infections

Immunity Associated with Bacterial Infections

Adaptive Immunity: Cellular vs. Humoral

CELLULAR	HUMORAL
T Cell / Lymphokines	B Cell (*Plasma Cell*) / Antibody
Primary Defense Against Viral / Fungal Infections (*Intracellular Organisms*)	Defense Against Bacterial Infections (*Extracellular Organisms*)
Hypersensitivity Type IV (*delayed*) (*Ex. Transplant Rejection*)	Hypersensitivity Type I (*Immediate*) Hypersensitivity Type II (*ADCC*) Hypersensitivity Type III (*Immune Complex*)

CHARACTERISTICS OF IMMUNOGENS (ANTIGENS) THAT AFFECT IMMUNOGENICITY

1. Large size molecule *(molecular weight of at least 10 KDa)*
2. High complexity *(simple repeating units do not make good immunogens)*
3. Chemical composition *(proteins and polysaccharides are better than carbohdrates, lipids & nucleic acids)*
4. Foreignness *(the more different from the host, the more immunogenic)*

CLASSES OF IMMUNOGLOBULINS (Based on Heavy Chains)

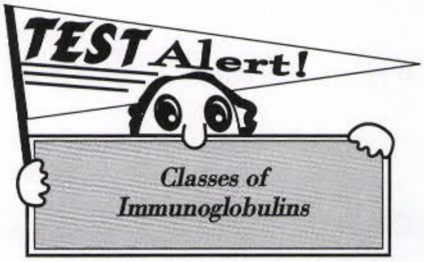
1. IgG
   a. Greatest concentration in serum
   b. 4 subclasses
   c. Activates complement *(except for IgG4)*
   d. Crosses placenta
   e. 75% of total antibody concentration

2. IgM
    a. Largest antibody *(pentamer)*
    b. Fixes complement best (multiple binding sites)
    c. Prominent in early immune response (indicates acute infection)
    d. 5-10% of total antibody concentration

3. IgA
    a. Predominant antibody in body secretions *(tears, saliva, nasal mucosa)*
    b. Serum IgA *(monomer)* & secretory IgA *(dimer)*
    c. Primary defense against local infections at mucosal surface
    d. Two subclasses

4. IgD
    a. Unknown function
    b. Present on B cell surface

5. IgE
    a. Allergy
    b. Type I hypersensitivity
    c. Involved in release of histamines from mast cells



REMEMBER!

Antibody Functions

Ig **G** - **G**reatest Plasma Concentration (approximately 70%); **G**oes Across Placenta

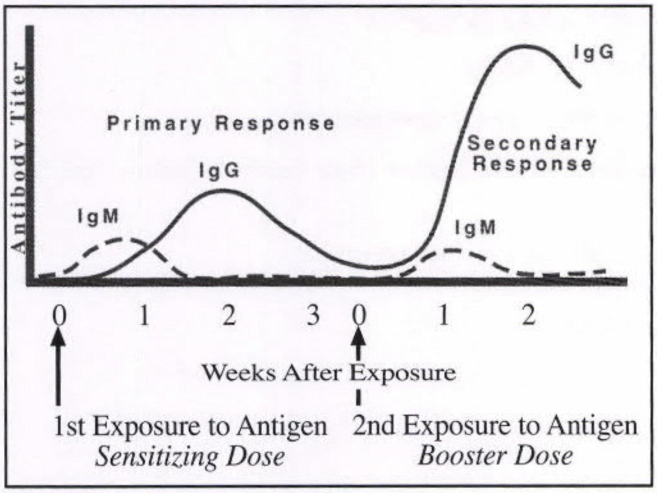
Ig **M** - **M**ega (largest immunoglobulin molecule); Activates Co**M**plement Easily

Ig **A** - S**A**liva, Te**A**rs (body secretions)

Ig **D** - **D**on't Know Function

Ig **E** - Allerg**EE** (allergy)

Immune Response Curve

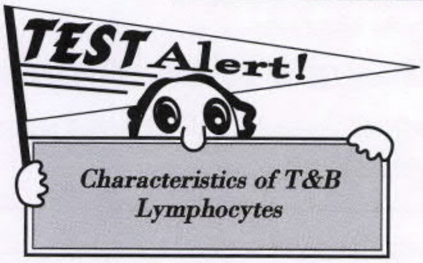


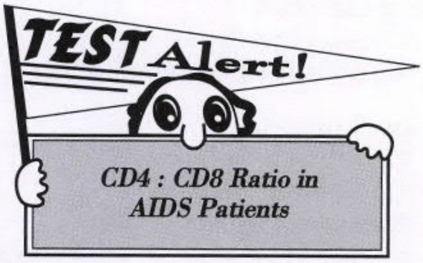
EVALUATION OF T AND B CELLS

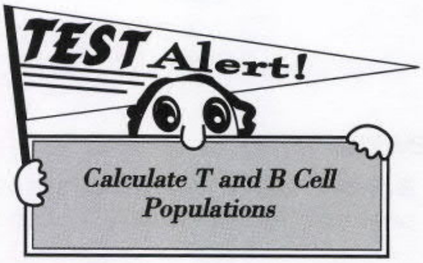
1. Historical method-Rosette Test
    a. Incubate sheep red blood cells *(SRBC)* with known number of purified lymphocytes; SRBCs bind to E-rosette receptor *(CD2)* on T cells
    b. Count rosetted lymphocytes; percentage of T cells can be calculated
    c. Estimate of B cells: 100% minus calculated percentage of T cells
    d. Estimates of absolute counts: patient's total lymphocyte count multiplied by percentage of T or B cells

Lymphocytes in the Immune Response			
	T cells	**B cells**	**Null Cells (NK,K)**
Description	Small lymphocyte	Small lymphocyte	Large lymphocyte
% of Total Circulating Lymphoid cells	≈ 80%	5 - 15%	5 - 15%
Surface Receptor	T cell receptor (TCR-1 or TCR-2)	Surface Immunoglobulin (IgD or IgM) Complement Receptors	No TCR No surface immunoglobulin
Surface Markers	CD2+ : Rosette with SRBCs CD3+ : Associated with TCR CD4+ : T Helper cell CD8+ : T cytotoxic/ regulatory cells	CD19+ CD20+ CD21+	CD16+ CD56+
Function(s)	CD4: Release cytokines Become memory cell CD8: Become Cytotoxic CD4 or CD8: Interact with B cells	Evolve into plasma cells which secrete antibody Become memory cell	Important in killing virus infected cells and tumor cells
Primary Immune Protection	Immune response against viral infections and tumors Important in delayed hypersensitivity reactions	Antibodies neutralize toxins, activate complement, and act as opsonins	• Natural Killer cells - cytotoxic without MHC restriction • Killer cells - Antibody Dependent Cellular Cytotoxicity (ADCC) Important in killing virus infected cells and tumor cells

Normal T cell : B cell Ratio = 8 : 1
Normal T helper : T regulatory Ratio = 2 : 1
AIDS Patient   Inverse T helper : T regulatory Ratio = 1 : 2



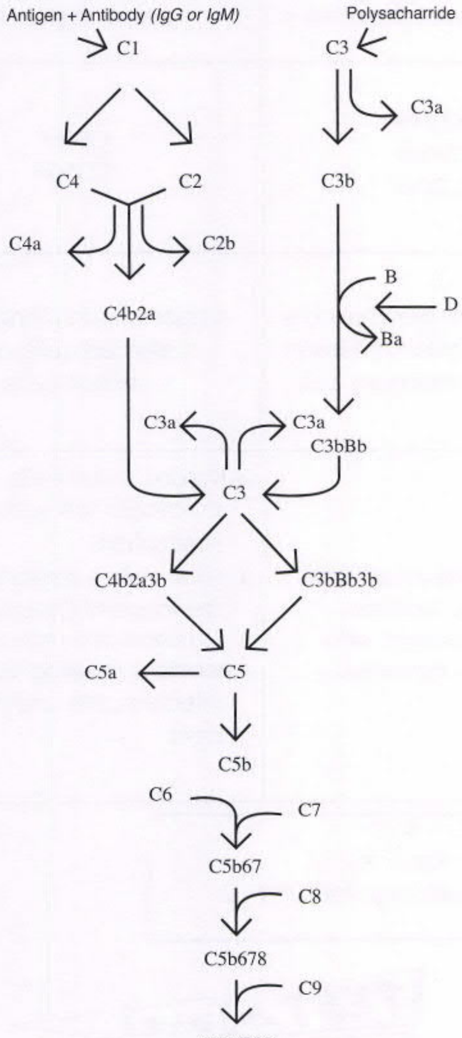

TEST Alert!
CD4 : CD8 Ratio in AIDS Patients


TEST Alert!
Calculate T and B Cell Populations

2. Monoclonal Antibodies
   a. Can be used to differentiate T and B cells by detecting cell surface markers *(CD markers)* with flow cytometry, ELISA or immunofluorescence

b. Produced by immunizing mouse with specific antigen, combining mouse spleen cells with myeloma cells *(plasma cells fuse with myeloma cells forming a hybridoma).* Hybridoma can produce monoclonal antibodies for indefinite period of time.

Complement Cascades


Antigen + Antibody *(IgG or IgM)*
C1
Polysacharride
C3
C3a
C4
C2
C3b
C4a
C2b
B
D
C4b2a
Ba
C3a
C3a
C3bBb
C3
C4b2a3b
C3bBb3b
C5a
C5
C5b
C6
C7
C5b67
C8
C5b678
C9
C5b6789

COMPLEMENT

1. Approximately 21 chemically distinct proteins *(14 effector, 7 control)*
2. Functions to control inflammation
   a. Activates phagocytes *(chemotaxis)*
   b. Lyses target cell *(foreign organism)*
   c. Opsonization - enhances phagocytic binding by coating foreign organism and attaching to complement receptors on neutrophils and monocytes

REMEMBER!

Complement Cascades

CLASSICAL

Components

- **Activated by Immune Complexes (*IgG, IgM*)**
- **Bind in Numerical Order Except at the Beginning (*C1, C4, C2, C3, etc.*)**
- **Usually "a" fragments go into plasma, "b" fragments attach to cell (*exception: C2a & C2b*)**

ALTERNATIVE

Factors

- **Activated by Lipopolysaccharides, Polysaccharides**
- **Involves C3 at 2 Points in Cascade**
- **Involves Factors B & D and Control Factors H&I**

3. Cascades (*pathways*) - require calcium and magnesium
   a. Classical pathway
   b. Alternative pathway
4. Control proteins
   a. C1 esterase inhibitor
   b. C4 binding protein
   c. Factor I *(degrades C3b)*
   d. Factor H *(competes with Factor B)*

Characteristics of Hypersensitivity Reactions

TYPE	MECHANISM	EXAMPLES
Type I (anaphylactic, immediate)	IgE Mediated (antigen binds to IgE-sensitized Mast Cell ➔ Histamine Released)	Bee Sting Hay Fever Asthma
Type II (antibody dependent cytotoxicity)	Antibody Attaches to Cell Bearing Corresponding Antigen ➔ Cell Death	Transfusion Reaction Autoimmune Hemolytic Anemia (AIHA) Hashimoto's Thyroiditis Goodpasture's Disease
Type III (immune complex)	Formation of Large Immune Complexes Not Cleared by Mononuclear Phagocytic System	Rheumatoid Arthritis (RA) Sytemic Lupus Erythematosus (SLE) Serum Sickness
Type IV (delayed)	Sensitized T Cells Release Interleukins; Monocyte and Lymphocyte Infiltration; > 12 hours to develop	Contact Dermititis (Poison Ivy, Chemicals) TB Leprosy Graft vs. Host Disease (GVHD)

Tests for Allergy: Skin Tests, RIST, RAST

ELISA for total IgE and IgE to specific antigens

Association of Eosinophils and IgE with Hypersensitivity Type I

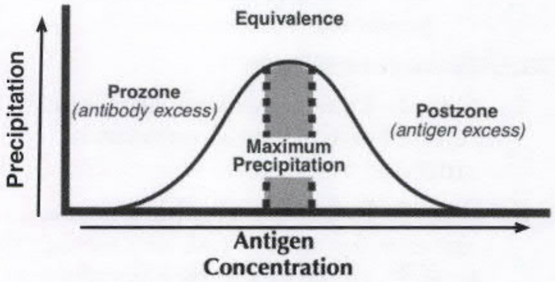
Principles of Antigen-Antibody Reactions Used in Serologic Testing

PRECIPITATION

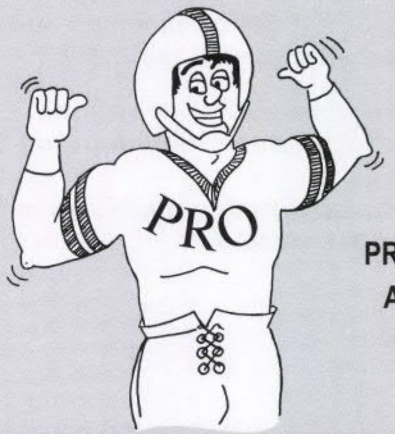
1. Principle
    a. Soluble antigen + antibody *(in proper proportions)*
    b. Lattice formation *(antigen binds with Fab sites of two antibodies)* ➔ visible precipitate

2. Examples
    a. Double diffusion (*Ouchterlony*)
    b. Single diffusion *(radial immunodiffusion)*
    c. Immunoelectrophoresis
    d. Immunofixation

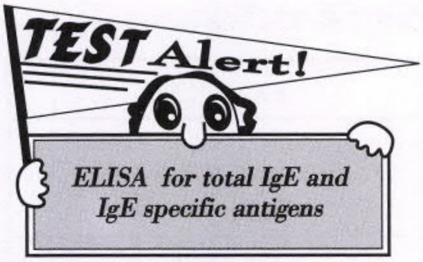
Precipitin Curve



REMEMBER!
Pros Have
Good Bodies



PROZONE =
Anti*body*
Excess



ELISA for total IgE and IgE specific antigens

AGGLUTINATION

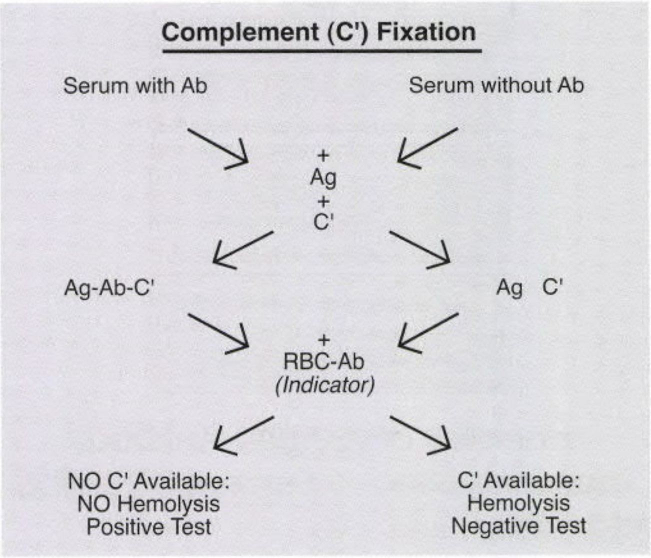
1. Principle
   a. Particulate antigen + antibody
   b. Lattice formation *(antigen binds with Fab sites of two antibodies forming bridges between antigens)* ➔clumping
2. Examples
   a. Direct agglutination *(Blood Bank)*
   b. Passive hemagglutination *(antigen reagent produced by treating RBCs with tannic acid to allow adsorption of protein antigens)*
   c. Passive latex agglutination *(antigen in reagent is attached to latex particle)*

AGGLUTINATION INHIBITION

1. Step 1: Patient serum (*antigen*) is reacted with limited amount of antibody reagent
2. Step 2: Indicator is added (*same antigen for which you are testing bound to RBC or latex carrier particle)*
3. **Positive test (*No agglutination*)**: If patient has antigen for which you are testing, the reagent antibody will be bound in step 1 and unavailable to react with the indicator
4. **Negative test (*Agglutination*)**: If patient does not have the antigen, reagent antibody is not bound in step 1 and is available to react with indicator
5. Examples of inhibition reactions:
   a. Hemagglutination inhibition test for rubella
   b. Latex agglutination inhibition test for other viruses

COMPLEMENT FIXATION (CF)

1. Step 1: Antibody and antigen allowed to combine in presence of complement
2. Step 2: Indicator is added *(SRBC coated with hemolysin)*
3. **Positive test *(No hemolysis):*** If complement is fixed in step 1, it will not be available to combine with indicator ➔ no hemolysis occurs
4. **Negative test (*Hemolysis*):** complement was not bound in step 1 and is available to react with indicator ➔ hemolysis occurs
5. Limitations
   a. Serum *must* be heat inactivated
   b. Stored serum becomes anticomplementary
   c. Elaborate QC and standardization required
   d. Only used for IgM antibodies



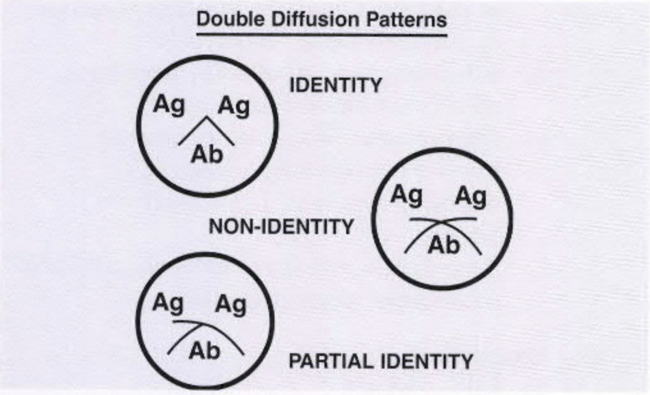
RADIAL IMMUNODIFFUSION (RID)

1. Also called single immmunodiffusion
2. Principle:
   a. Unlimited antibody incorporated into agar in plate
   b. Serum and standards are added to circular wells precut in agar
   c. Incubate
   d. Diffusion occurs and ring of precipitate forms
   e. Measure diameter (d) of ring

2. Methods
    a. Fahey (kinetic)
        - *Read before ring reaches maximal size (6-12 hours)*
        - *Logarithmic relationship between diameter (d) of precipitin ring and antigen concentration → read from plotted standard curve*
    b. Mancini (end-point)
        - *Read at maximal size (24-48 hours)*
        - *Linear relationship between diameter squared (d^2) of precipitin ring and antigen concentration → read from plotted standard curve*

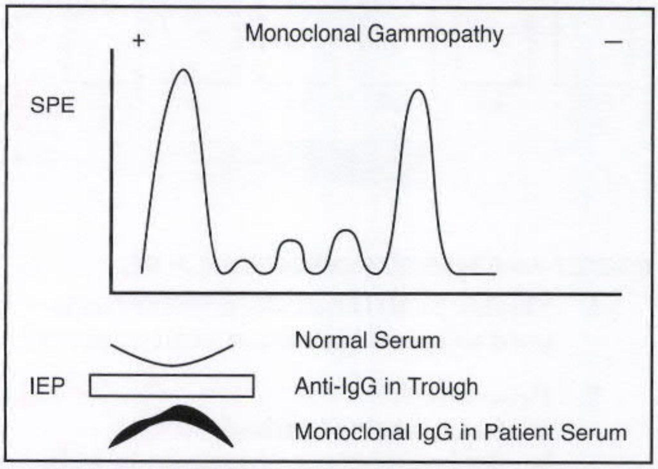
DOUBLE DIFFUSION (OUCHTERLONY)

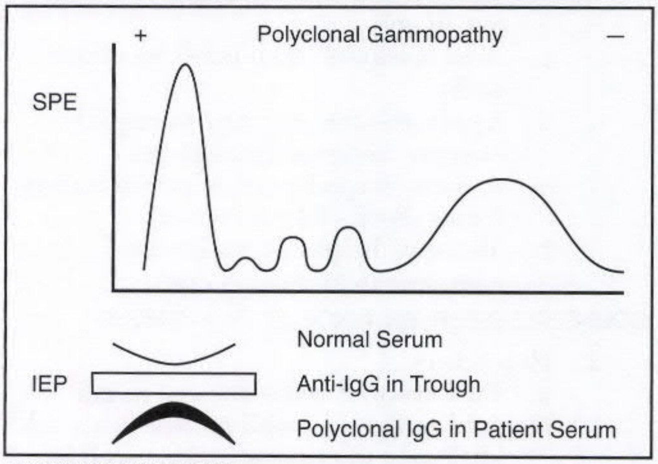
1. Used to determine relationships between antigens and antibodies
2. Antibody is added to precut wells in center of agar plate (Agar contains no antigen or antibody)
3. Patient sera and standards are alternated in wells surrounding the center well (*antibody well*)
4. Incubate
5. Diffusion occurs and results in visible bands of precipitation
6. Patient wells are read in relation to standards in adjacent wells (see patterns below)
7. Location of bands depends on concentration and rate of diffusion
8. Used to identify antibodies associated with autoimmune disorders


Double Diffusion Patterns
IDENTITY
Ag
Ag
Ab
NON-IDENTITY
Ag
Ag
Ab
Ag
Ag
Ab
PARTIAL IDENTITY

IMMUNOELECTROPHORESIS (IEP)

1. Gel diffusion + electrophoresis
2. Electrophorese serum proteins on agar gel
3. Fill trough in agar with known antibody
4. Antigen and antibody diffuse through agar
    a. In equivalence zone, precipitation arc appears
    b. Size of arc determined by antigen concentration
    c. Abnormal contour of arc may indicate monoclonal gammopathy
5. Most commonly used to determine heavy and light chains involved
    a. Serum IEP: monoclonal gammopathies
    b. Urine IEP: Bence Jones protein

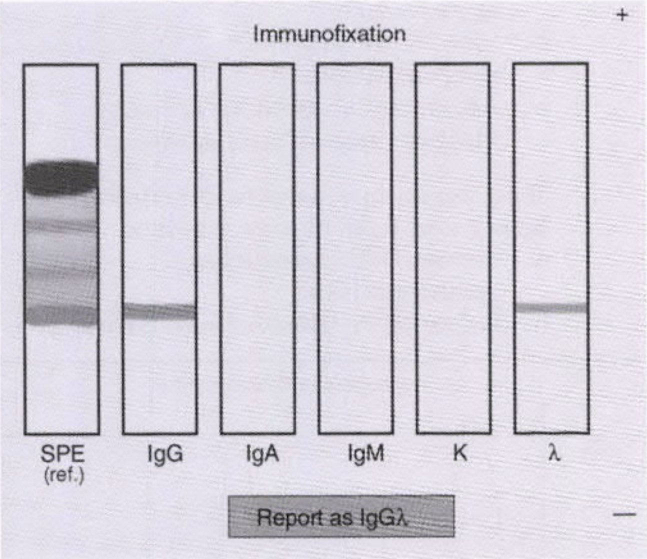

Monoclonal Gammopathy
+
–
SPE
IEP
Normal Serum
Anti-IgG in Trough
Monoclonal IgG in Patient Serum


Polyclonal Gammopathy
+
–
SPE
IEP
Normal Serum
Anti-IgG in Trough
Polyclonal IgG in Patient Serum

IMMUNOFIXATION

1. Protein electrophoresis + immunoprecipitation
2. Procedure
    a. Apply specimen to 6 positions on agarose plate
    b. Electrophorese to separate proteins
    c. Apply monospecific antisera to 5 lanes using a 6th for reference
    d. If antigen present, band of antigen-antibody complexes form and precipitate; wash, stain

3. Highly sensitive method, easy to read
4. Used to classify monoclonal gammopathies *(determine heavy and light chains involved)*



ROCKET IMMUNOELECTROPHORESIS (LAUREL)

1. Similar to RID but electrophoresis is used to speed formation of precipitate
2. Procedure
    a. Gel contains antibody
    b. Add patient sera (*antigen*) to wells cut in gel
    c. Add assayed standards to other wells
    d. Apply electric current to rapidly migrate antigen through gel
    e. A cone-shaped area of precipitation forms *(looks like a rocket)*
    f. Measure height of rocket and compare to standard curve

COUNTERCURRENT IMMUNOELECTROPHORESIS

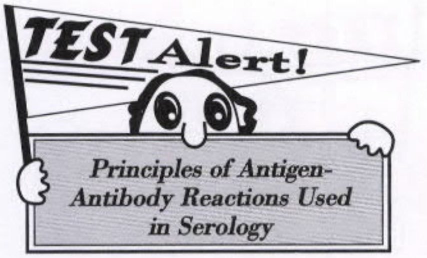
1. Procedure
    a. Two rows of wells are cut in gel
    b. Add antigen to well in one row; add antibody to corresponding well in other row (*one known, the other unknown*)
    c. Apply electric current to gel
    d. Antigen and antibody migrate toward each other
    e. Precipitate forms if specific antigen for the antibody in the corresponding well is present in patient's serum

RADIOIMMUNOASSAY (RIA)

1. Very sensitive and specific
2. Can be used for detecting antigen or antibody *(explanation below detects antigen, but using known antigen to detect serum antibody is also possible)*
3. Competitive binding assay
    a. Patient antigen and labeled antigen are incubated with known amount of specific antibody *(unlabeled and labeled antigen compete for binding with antibody)*
    b. Wash to remove unbound antigen
    c. Radioactivity counted on a gamma counter; compare to standard curve
    d. Results
        - *The lower the radioactive count, the higher the concentration of unlabeled antigen (patient)*
4. Examples
    a. Tests for hepatitis antigens and antibodies
    b. Radioimmunosorbent Test (*RIST*) - measures total IgE
    c. Radioallergosorbent Test (*RAST*) - measures IgE to specific allergens

ENZYME IMMUNOASSAY (EIA/ELISA)

1. " Sandwich technique"
    a. Monoclonal or polyclonal antibody adsorbed on solid surface *(bead or microtiter well)*
    b. Add patient serum; if antigen is present in serum, it binds to antibody-coated bead or well
    c. Add excess enzyme-labeled antibody *(antibody conjugate);* forms antigen-antibody-labeled antibody "sandwich" *(antibody in conjugate is directed against another epitope of antigen being assayed)*
    d. Add enzyme substrate, incubate and read absorbance
    e. **Important**: Washing required between each step. Improper washing leads to false positive results
    f. Absorbance is directly proportional to antigen concentration
2. Examples:
    a. HIV testing
    b. Serum HCG *(pregnancy)*
    c. Tests for hepatitis antigens and antibodies
    d. Antibodies to bacteria and viruses



Principles of Antigen-Antibody Reactions Used in Serology

ENZYME MULTIPLIED IMMUNOASSAY (EMIT)

1. Used to measure concentrations of small molecules such as drugs and hormones
2. Principle
   a. Add patient serum to an enzyme-drug conjugate; then add anti-drug antibody (*reagent*)
   b. Add enzyme substrate and incubate
   c. Positive test
      - ❖ *Drug in patient serum combined with anti-drug antibody; sites on the enzyme portion of the conjugate remain available to bind with substrate*
      - ❖ *Color produced*
   d. Negative test
      - ❖ *Anti-drug antibody attached to the drug in the enzyme drug conjugate and blocks the active sites on the enzyme; substrate unable to bind to enzyme*
      - ❖ *No color produced*

NEPHELOMETRY

1. Procedure
   a. Serum substance reacts with specific antisera and forms insoluble complexes
   b. Light is passed through suspension
   c. Scattered *(reflected)* light absorbance is proportional to number of insoluble complexes; compare to standards

2. Examples:
   a. Complement component concentration
   b. Antibody concentration *(IgG, IgM, IgA, etc.)*

IMMUNOFLUORESCENCE

1. Direct — Add fluorescein-labeled antibody to patient tissue, wash & examine under fluorescent microscope

2. Indirect (IIF)— Add patient serum to reagent (*tissue containing known antigen*), wash, add fluorescein labeled antiglobulin, wash & examine under fluorescent microscope

3. Examples of IIF
   a. Testing for Antinuclear Antibodies *(ANA)*
   b. Fluorescent Treponemal Antibody Test *(FTA-Abs)*

FLUORESCENCE POLARIZATION IMMUNOASSAY (FPIA)

1. Principle
   a. Add reagent antibody and fluorescent-tagged antigen to patient serum
   b. Positive test
      - ❖ *Antigen present in patient serum binds to reagent antibody leaving most tagged antigen unbound*
      - ❖ *Unbound tagged antigens rotate quickly reducing amount of polarized light produced*
   c. Negative test
      - ❖ *If no antigen present in patient serum, tagged antigen binds to reagent antibody*
      - ❖ *Tagged antigen antibody complexes rotate slowly giving off increased polarized light*

FLOW CYTOMETRY

See Lab Operations and Instrumentation Chapter for explanation

SERIAL DILUTIONS

1. Testing for infectious diseases is performed on acute and convalescent specimens collected about 2 weeks apart

2. Must see 4-fold or 2 tube rise in titer to be clinically significant

TERMS USED IN EVALUATING TEST METHODOLOGY

1. Sensitivity
   a. Analytical Sensitivity — Ability of a test to detect very small amounts of a substance
   b. Clinical Sensitivity — Ability of test to give positive result if patient has the disease - 100% clinical sensitivity gives no false negative results

N is for **N**egative

se**N**sitivity (%)= TP/(TP+F**N)** X100

(***False negatives yield lower sensitivity***)

P is for **P**ositive

s**P**ecificity (%)= TN/(TN+F**P**) x 100

(False positives yield lower specificiity)

TEST Alert!

Serial Dilution Calculations

Compare Pre & Post Titers to Determine Diagnosis of Measles and Rubella

Definitions for Sensitivity and Specificity

2. Specificity
   a. Analytical Specificity — Ability of test to detect substance without interference from cross-reacting substances
   b. Clinical Specificity — Ability of test to give negative result if patient does not have disease - high clinical specificity test gives no false positives

REMEMBER!

Test Sensitivities

Nonlattice (More Sensitive)

Immunoassays
- •RIA *(Radial Immunoassay)*
- •EIA *(Enzyme Immunoassay)*
- •FIA *(Fluorescent Immunoassay)*

Nephelometry

Lattice (Less Sensitive)

CIE *(Counter Current Immunoelectrophoresis)*

(..."tion" or ..."sion")
- •CF *(Complement Fixation)*
- •Agglutination
- •Flocculation *(Precipitation)*

Rocket Electrophoresis

RID *(Radial Immunodiffusion)*

Ouchterlony *(Double immunodiffusion)*

IFE *(Immunofixation)*

IEP *(Immunoelectrophoresis)*

0.001mg/ml

Sensitivity

500 mg/ml

TEST Alert!

Relative Sensitivities of Serologic Methods

Disease States and Associated Laboratory Tests

INFLAMMATION

1. C-reactive protein (acute phase protein)
   a. Released rapidly after inflammation or tissue damage
   b. Concentration quickly decreases as inflammation subsides
   c. Used to monitor presence of inflammation
   d. Does not indicate source of inflammation
   e. High sensitivity CRP (*hs-CRP*) used to indicate risk of coronary artery disease

2. Erythrocyte sedimentation rate (*ESR*)
   a. Also used to indicate presence of inflammation
   b. Not as sensitive to increasing or decreasing inflammation as CRP

SYPHILIS

1. Caused by *Treponema pallidum*

2. Course of disease: Primary, secondary, latent, tertiary; also congenital infections may occur

TREPONEMAL TESTS

1. Darkfield microscopy - used to visualize motile organisms from primary & secondary lesions

2. Fluorescent treponemal antibody absorption test *(FTA-Abs)*
   a. Indirect immunofluorescence assay
   b. Remove nonspecific antibodies from serum by using sorbent
   c. React serum with Nichol's strain of *T. pallidum*
   d. Add fluorescein-labeled antihuman globulin and wash
   e. Read for fluorescence

3. *Treponema pallidum* Immobilization Test *(TPI)*
   a. Darkfield microscopy
   b. Add live treponemes to patient serum
   c. If antibody is present, treponemes immobilized
   d. Expensive, seldom used

4. Microhemagglutination Assay for *T. pallidum (MHA-TP)*
   a. Add patient serum to red cells sensitized with *T. pallidum*
   b. If antibody is present, agglutination occurs

NONTREPONEMAL TESTS (REAGIN TESTS)

1. Venereal Disease Research Laboratory *(VDRL)*
   a. Microflocculation (*microscopic*)
   b. Antigen = cardiolipin + lecithin
   c. Antibody *(reagin)* = IgM or IgG directed against damaged tissue or organism
   d. Serum requires heat inactivation
   e. Flocculation indicates reactive serum
   f. Test of choice for screening CSF
   g. False positive in malaria *(100% biologic false positive)*, SLE, RA, hepatitis, pneumonia, aging and infectious mononucleosis

2. Rapid Plasma Reagin Test *(RPR)*
   a. Microflocculation and coagglutination of charcoal particles *(macroscopic)*
   b. More sensitive, less specific than VDRL
   c. Antigen = cardiolipin + charcoal particles
   d. No heat inactivation necessary
   e. Black clumps form in reactive test
   f. False positives — same as VDRL

REMEMBER!

Do NOT Refrigerate Specimen for Cold Agglutinin Assay

Antibody will bind to red cells leaving serum free of antibodies and result in a false negative or decreased cold agglutinin titer.

Sensitivity of Tests for Syphilis

PRIMARY STAGE

FTA-ABS
RPR } ↑ Sensitivity
VDRL

SECONDARY STAGE

All Tests Equally Sensitive

LATE STAGE

FTA-ABS
MHA-TP } Equal Sensitivity
TPI

Reagin Tests — Negative in treated patients

FTA-ABS: *Most Sensitive in All Stages*

RHEUMATOID ARTHRITIS (RA)

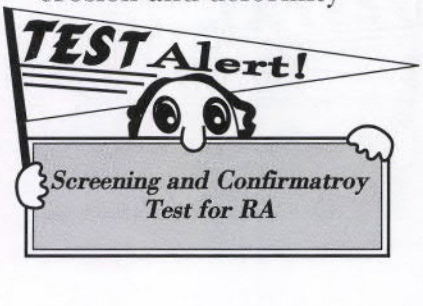
1. Production of IgM or IgG antibodies directed against IgG

2. Diagnosis requires radiologic, clinical and laboratory findings

3. Laboratory findings
   a. High titers of rheumatoid factor (*RF*)
   b. Low titers of complement
   c. Positive anti-cyclic citrullinated peptide (*anti-CCP*)

4. Screening test: Rheumatoid factor (*RF*) assay
   a. Patient serum added to reagent composed of particulate carrier (*latex or RBC*) attached to IgG
   b. Run positive and negative controls
   c. Positive test: visible agglutination
   d. Detects serum IgM

5. Confirmatory test: Anti-cyclic citrullinated peptide assay (*anti-CCP*)
   a. Used to confirm positive RF tests
   b. ELISA technique
   c. High sensitivity and specificity
   d. Allows earlier diagnosis and earlier treatment which may reduce joint erosion and deformity



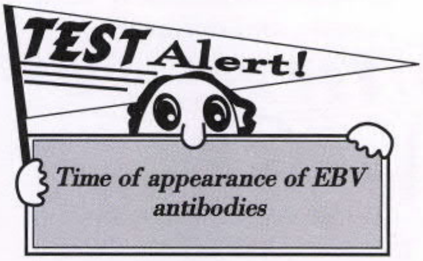
Screening and Confirmatroy Test for RA

CELIAC DISEASE (CELIAC SPRUE)

1. Hypersensitivity to gliadin (*a protein component of gluten*) found in grains such as wheat, barley, rye
2. Laboratory findings
   a. Tissue transglutaminase antibody *(tTG-IgA, tTG-IgG)* currently considered best tests for celiac disease
   b. Endomysial antibody (*EMA-IgA*)
   c. Antigliadin antibody (*AGA-IgG, AGA-IgA*)

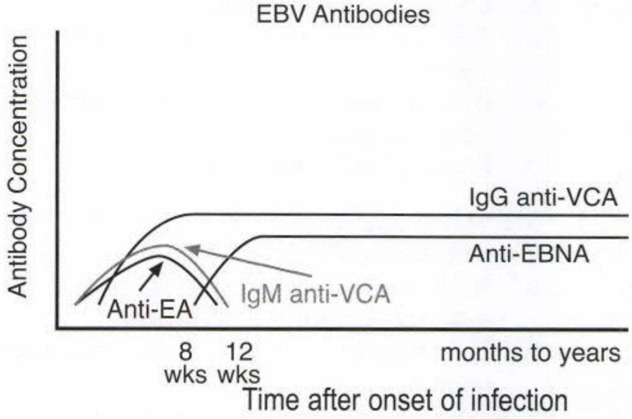
INFECTIOUS MONONUCLEOSIS (I.M.)

1. Causative agent: Epstein-Barr Virus *(EBV)*
2. Screening tests for heterophile antibodies
   a. Rapid Differential Slide Test
      - ❖ *Step I: Using two serum aliquots, absorb one with kidney cells (guinea pig or horse) and the other with beef erythrocytes*
      - ❖ *Step II: React each aliquot with sheep or horse RBCs (indicator)*
      - ❖ *Positive for I.M: Greater agglutination in kidney absorbed serum than in beef RBC absorbed serum*
      - ❖ *Run positive and negative controls*
      - ❖ *False positives: leukemia, CMV, Burkitt's lymphoma, RA, viral hepatitis*
   b. Rapid ELISA Slide Test
3. Confirmation test: EBV specific antibody tests
   a. Immunofluorescent assay or ELISA for IgM and IgG anti-viral capsid antigen *(anti-VCA, IgM and anti-VCA, IgG)*, anti-early antigen *(anti-EA)*, and anti- nuclear antigen *(anti-EBNA)*
   b. Appearance and duration of antibodies are used to differentiate acute from past infection
   c. IgG or IgM anti-VCA in absence of anti-EBNA indicates current or recent IM infection



Time of appearance of EBV antibodies

EBV Antibodies



EBV Specific Serology

METHOD:
Indirect Immunofluorescence or ELISA

RECENT OR CURRENT INFECTION
IgM Anti-VCA
Anti-EA
IgG AntiVCA *without* Anti-EBNA

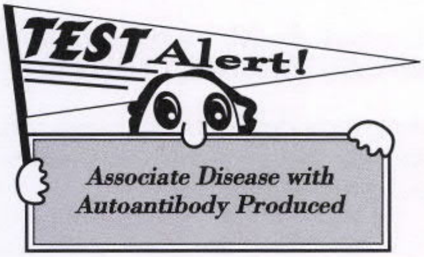
PAST INFECTION
Anti-EBNA
IgG Anti-VCA *without* IgM Anti-VCA

STREPTOCOCCUS (GROUP A) INFECTION

1. Streptozyme
   a. Slide agglutination test used to screen for antibodies to 5 streptococcal antigens *(streptolysin, streptokinase, DNase, NADase, and hyaluronidase)*
   b. Procedure
      - ❖ *Reagent = Sheep red blood cells (SRBC) coated with the 5 streptococcal antigens*
      - ❖ *Dilute patient serum and add to SRBC reagent*
      - ❖ *If antibodies to any of these antigens are present, hemagglutination occurs*
2. Anti DNase-B Test
   a. Highly specific neutralization test for anti-DNase produced in group A *Streptococcus* infection
   b. Better than ASO test for cases of glomerulonephritis *(anti-streptolysin O is often not produced in glomerulonephritis)*

c. Principle — DNase B reagent hydrolyzes DNA-methyl green conjugate reagent changing the color from green to colorless

d. Procedure
- ❖ *Add patient serum to DNase B reagent and incubate*
- ❖ *Add DNA-methyl green conjugate*
- ❖ *If anti-DNase B is present in the patient's serum, DNase B reagent is neutralized and unable to decolorize the conjugate; green color persists and is graded from 2+ to 4+*


TEST Alert!
Associate Disease with Autoantibody Produced

SLE, SJOGREN'S SYNDROME, SCLERODERMA, RA, ETC

1. Autoimmune diseases

2. Tests for Antinuclear Antibodies (*ANA*)
   a. ELISA-usually used to screen
   - ❖ *Easier to perform and interpret than immunofluorescence*
   - ❖ *Less costly*

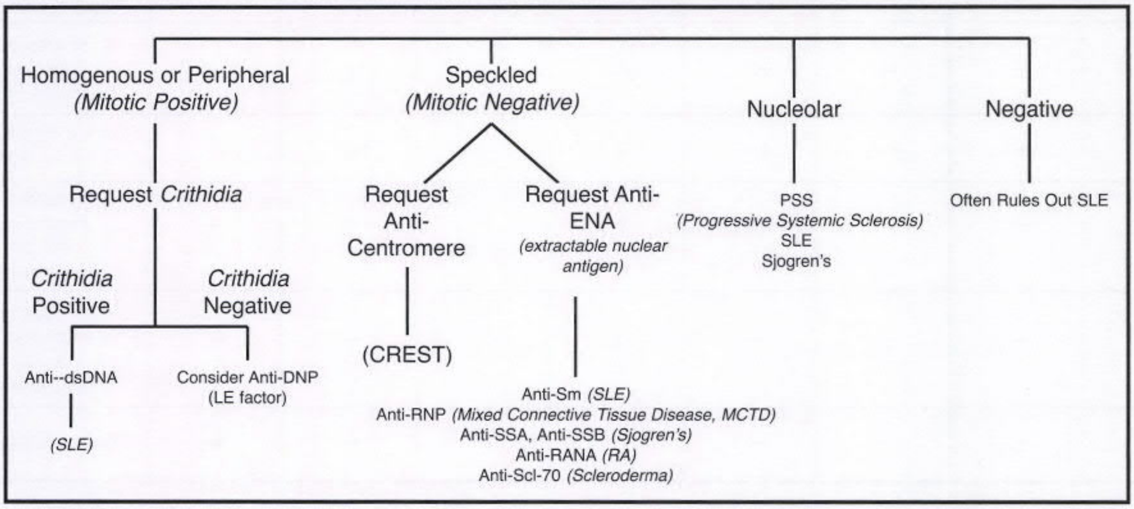
   b. Indirect immunofluorescence (*IIF*)
   - ❖ *Add dilutions of patient serum to tissue nuclei of human epithelial cells (HEp2 cells) attached to a slide*
   - ❖ *Wash and add fluorescent-labeled antihuman globulin (AHG)*
   - ❖ *Read pattern under fluorescent microscope; report titer and pattern (see table for disease associated patterns)*

   c. Confirmation (*see chart below*)

Antinuclear Antibodies

ANA Pattern	Antibody	Disease
Homogeneous *(diffuse)*	Anti-histone	usually SLE
Peripheral (Rim)	Anti-ds DNA *(double-stranded DNA)*	SLE
Speckled	Anti-RNA Anti-ENA *(extractable nuclear antigens)*	SLE Scleroderma (*PSS*) RA MCTD
Nucleolar	Anti-nucleolar RNA	Scleroderma Sjogren's

Testing for Antinuclear Antibodies


Homogenous or Peripheral
(Mitotic Positive)
Request Crithidia
Crithidia Positive
Crithidia Negative
Anti--dsDNA
(SLE)
Consider Anti-DNP (LE factor)
Speckled
(Mitotic Negative)
Request Anti-Centromere
(CREST)
Request Anti-ENA
(extractable nuclear antigen)
Anti-Sm (SLE)
Anti-RNP (Mixed Connective Tissue Disease, MCTD)
Anti-SSA, Anti-SSB (Sjogren's)
Anti-RANA (RA)
Anti-Scl-70 (Scleroderma)
Nucleolar
PSS
(Progressive Systemic Sclerosis)
SLE
Sjogren's
Negative
Often Rules Out SLE

Basic ANA Patterns

ANA Confirmatory Tests

Associate Autoimmune Disease with Specific Antibodies

ACQUIRED IMMUNODEFICIENCY (AIDS)

1. HIV-1 retrovirus attacks CD4+ cells *(T helper and macrophage)*
2. Clinical manifestations may include pneumococcus pneumonia, Kaposi sarcoma, recurrent infections
3. Lab tests
   a. Screen using ELISA procedure for HIV-1 antibody
   b. Confirm
      - Western Blot *(positive if bands for p24, gp41 and gp120 or gp160 are present)*
      - *Nucleic acid testing*

   c. T helper/T regulatory ratio is decreased

CYTOMEGALOVIRUS (CMV)

1. Herpes virus
   a. Usually causes asymptomatic infection
   b. May be serious in immunocompromised patients and infants receiving transfusions

2. Presence of antibody does not prevent reinfection

3. Test using ELISA procedure

VIRAL HEPATITIS

1. Inflammatory disease of the liver

2. Associated with increase in liver enzymes *(AST, ALT, GGT)*

3. Diagnosis depends on appearance of specific antigens and antibodies in serum (*see chart*)

Screening and Confirmatory Tests for HIV

Interpretation of Hepatitis B Testing

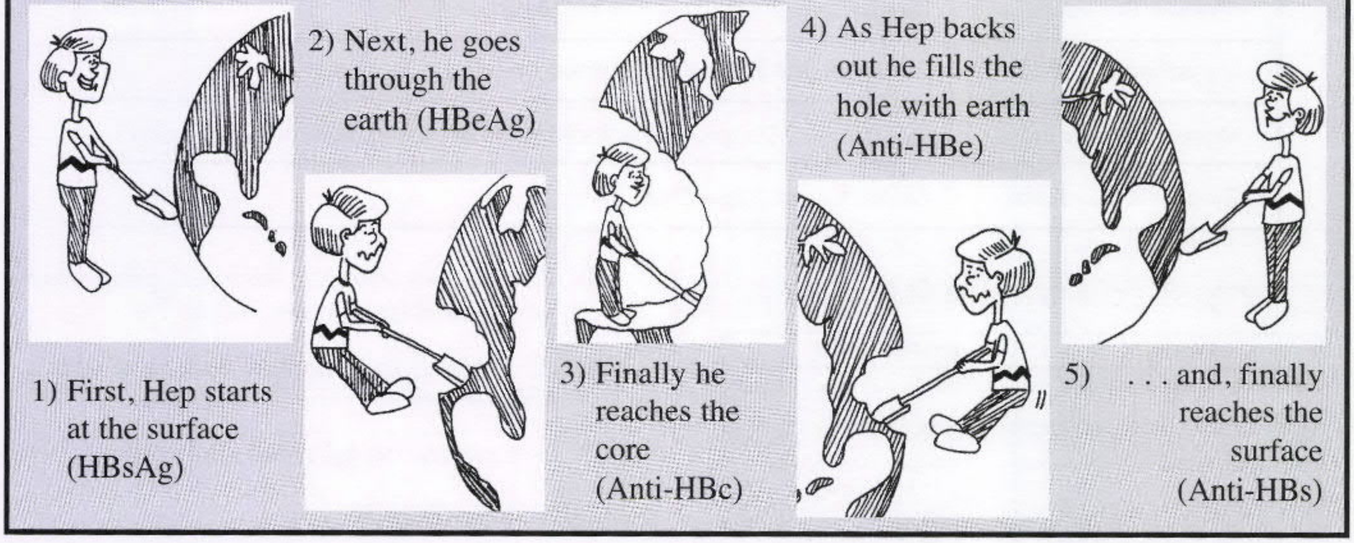
Serodiagnosis of Hepatitis

HBsAg	HBeAg	Anti-HBe	Anti-HBc *(IgM)*	Anti-HBc	Anti-HBs	Anti-HAV *(IgM)*	Anti-HCV	Interpretation
—	—	—	—	—	—	+	—	Recent Acute Hepatitis A Infection
+	+	—	+/—	+/—	—	—	—	Acute Hepatitis B Infection (highly infectious)
+	+	+/—	+/—	+	—	—	—	Chronic Hepatitis B / Carrier State
—	—	+	—	+	+	—	—	Immunity to Hepatitis B Due to Past Infection
—	—	—	—	—	+	—	—	Immunity to Hepatitis B Due to Vaccination
—	—	—	—	—	—	—	+	Hepatitis C Infection

REMEMBER!

Hepatitis (Hepatitis B Antigens & Antibodies)

Hep will help you remember the order of *Hepatitis B* antigens & antibodies!
Picture Hep with a shovel trying to dig to the center of the earth.



1) First, Hep starts at the surface (HBsAg)

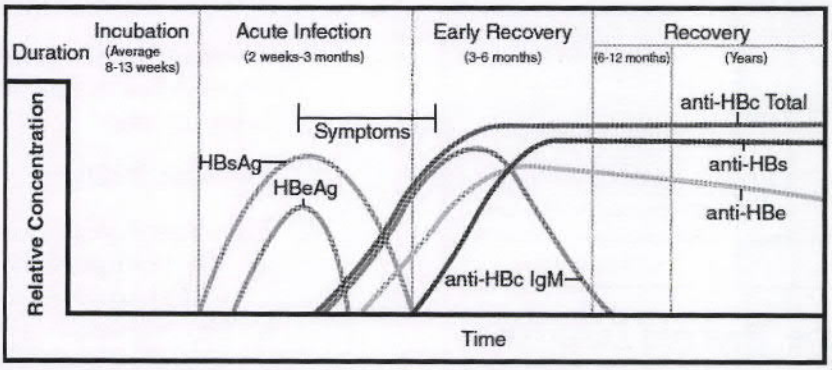
2) Next, he goes through the earth (HBeAg)

3) Finally he reaches the core (Anti-HBc)

4) As Hep backs out he fills the hole with earth (Anti-HBe)

5) . . . and, finally reaches the surface (Anti-HBs)

Acute Hepatitis B Diagnostic Profile



Source:CDC

Autoimmune Diseases

MULTIPLE SCLEROSIS (MS)

1. Autoantibodies to myelin sheath of nerves or myelin basic protein
2. Laboratory findings
   a. Oligoclonal IgG bands in CSF but not in serum *(indicates CNS production)*
   b. Usually IgG

TEST Alert!

Disease Associated with Oligoclonal IgG Bands in Spinal Fluid

TEST Alert!

Antibodies Associated with Autoimmune Diseases

Specificity of Autoantibodies

DISEASE	AUTOANTIBODY DIRECTED AGAINST:
Grave's Disease	Receptors for Thyroid Stimulating Hormone (TSH)
Goodpasture's Disease	Basement Membrane (kidney, lungs)
Hashimoto's Thyroiditis	Thyroglobulin
Multiple Sclerosis	Myelin Sheath of Nerves or Myelin Basic Protein
Myasthenia Gravis	Acetylcholine Receptors at Neuromuscular Junctions
Rheumatoid Arthritis	IgG (Fc) — 19s anti-IgM autoantibody known as Rheumatoid Factor
Sjogren's Syndrome	Salivary Duct / Tear Duct

Impaired Immune Function

DISEASE	DYSFUNCTION
Chronic Granulomatous Disease	Ineffective Phagocytosis
Chediak-Higashi Syndrome	Impaired Neutrophil Function
DiGeorge's Syndrome	T Cell Deficiency *(Absence of Thymus)*
Human Immunodeficiency Virus *(HIV)*	↓ T-Helper Cell ↓ Th/Ts Ratio ↓ T Cell Proliferation
Wiskott-Aldrich Syndrome	Partial Combined Immunodeficiency
Severe Combined Immunodeficiency Disease (SCID)	Complete or Marked Deficiency of T and B Lymphocytes

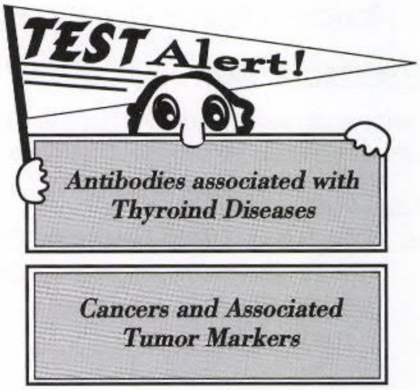
Autoimmune Thyroid Diseases

GRAVES DISEASE

1. Results in hyperthyroidism
2. Autoantibodies are directed against thyroid stimulating hormone receptors (*TSH-R*) on thyroid cells
3. Low TSH
4. Elevated thyroid antibodies
    a. Thyroid perioxidase antibody (*TPOAb or anti-TPO*)
    b. Thyroid stimulating hormone receptor antibody (*TRAb or TSHR Ab*)
    c. Thyroid stimulating immunoglobulin (*TSI*)
5. Antibodies lead to continual production of thyroid hormones, T3 & T4
6. Thyroid glands appear enlarged with diffuse goiter
7. Symptoms: weight loss, anxiety

HASIMOTO'S THYROIDITIS

1. Results in hypothyroidism (inadequate production of thyroid hormone)
2. Autoantibodies produced against thyroglobulin and thyroid cell components
3. Elevated TSH
4. Elevated thyroid antibodies
    a. Thyroid perioxidase antibody (*TPOAb or anti-TPO*)
    b. Thyroglobulin antibody (*TgAb or anti-Tg*); may also be present in thyroid cancer
5. Symptoms: weight gain, lethargy, intolerance to cold



Antibodies associated with Thyroind Diseases

Cancers and Associated Tumor Markers

CANCER

TUMOR MARKERS

1. **Substances synthesized and released by a tumor or produced by host in response to a tumor**
2. **Found in circulation, body cavity fluids, cell membranes or cytoplasm/nucleus of a cell**
3. **Absent or only trace amounts in normal population**
4. **Used in diagnosing, determining disease progression, choosing therapeutic drugs, monitoring response to therapy, and detecting recurrence**
    a. **Most common use is monitoring for tumor recurrence**
5. **Testing commonly performed by ELISA**

Tumor Markers

TUMOR MARKER	ASSOCIATED CANCER
Alpha Fetoprotein (AFP)	liver, ovary, testes (tetrablastoma)
Carcinoembryonic antigen (CEA)	colon, breast, lung
CA 15-1, BR 17.29, BR 27.29	breast
CA 125	ovary
CA 19-9	pancreas
Estrogen/ Progesterone receptors	breast
HER2/neu	breast - use of Herceptin
Prostate specific antigen (PSA)	prostate

IMMUNOLOGY PRACTICE QUESTIONS

1. A specimen is tested for antibodies to varicella resulting in a titer of 320. Two weeks later another specimen is drawn from the patient and the resulting titer is 640. A third test is done on a specimen drawn 4 weeks after the first specimen and the titer is 320. What is the disease status of the patient?
    A. **Antibody levels are increased but titers indicate a past infection with chicken pox.**
    B. **Antibody levels are within normal reference limits.**
    C. **Elevated antibody levels indicate a current chicken pox infection.**
    D. **The second titer indicates a current infection with chicken pox.**

2. A patient is immunized for rubella. What type of immunity does this patient have?
    A. **Active**
    B. **Passive**
    C. **Adoptive**
    D. **Natural**

3. An immunofluorescence procedure is performed to test for specific antibodies to Epstein Barr Virus. The following antibodies were found:

anti-VCA
anti-EA
anti-EBNA

How would these results be interpreted?
    A. **Early infection**
    B. **Recent infection**
    C. **Current infection**
    D. **Past infection**

4. ANA fluorescent techniques were performed and a speckled pattern appeared with a titer of 640. What would you do next?
    A. **Repeat the procedure.**
    B. **Test for extractable nuclear antibodies.**
    C. **Look for fluorescent mitotic cells.**
    D. **Test for DNA (Use *Crithidia luciliae* substrate).**

5. Multiple, homogeneous, narrow bands are present in the gamma zone on electrophoresis of a patient's CSF on agarous gel. Immunofixation indicates that the bands are primarily IgG. This may indicate which of the following diseases:
   A. Addison's disease
   B. Myasthenia gravis
   C. Multiple sclerosis
   D. Multiple myeloma

6. Assess the disease state of the patient with the following results:

HBsAg	positive
HBeAg	positive
anti-HBc	positive
anti-HBe	negative
anti-HBsAg	negative

   A. Incubation period for Hepatitis B infection
   B. Very early infection with Hepatitis B
   C. Highly infectious stage of Hepatitis B infection
   D. Immunity to Hepatitis B

7. After exposure to measles, a patient is tested and has a 1:20 titer. This indicates:
   A. Current infection
   B. Immunity
   C. Immunization
   D. Test should be repeated in 10 days to 2 weeks.

8. The best method for screening cerebrospinal fluid for syphilis is:
   A. VDRL
   B. RPR
   C. FTA-abs
   D. darkfield microscopy

9. A patient has a T helper:T regulatory ratio of 1:2. What disease state might you expect?
   A. infectious mononucleosis
   B. rheumatoid arthritis
   C. AIDS
   D. systemic lupus erythematosus

10. Multiple myeloma most commonly involves the following class of immunoglobulin:
   A. IgA
   B. IgD
   C. IgG
   D. IgM

11. To test for antibodies to specific allergens, the following procedure would be performed:
   A. RPR
   B. CRP
   C. RIST
   D. RAST
   E. VDRL

12. A patient suspected of having syphilis had various tests performed with the following results:

   Rapid plasma reagin (RPR) — reactive
   FTA-ABS — nonreactive
   VDRL (CSF) — nonreactive

   These test results best reflect which of the following?
   A. neurosyphilis
   B. biologic false positive
   C. primary stage syphilis
   D. secondary stage syphilis
   E. tertiary stage syphilis

13. An agglutination procedure is performed with the following results:

1:10	1:20	1:40	1:80	1:160	1:320
-	+	+	+	+	-

The reportable titer for this test is:

   A. 40
   B. 160
   C. 320
   D. Results are inconsistent. Procedure should be repeated.

14. Listed below are the results for a patient who had a positive ANA on initial testing.

Anti-Sm	negative
Anti-SSA	positive
Anti-SSB	positive
Anti-Scl-70	negative
Anti-RANA	negative

   The disease most closely associated with these results is:
   A. SLE
   B. mixed connective tissue disease
   C. scleroderma
   D. Sjogren's syndrome

15. The tumor marker associated with cancer of the pancreas is:

    A. CEA
    B. CA 19-9
    C. CA 125
    D. CA 15-3

16. The most sensitive assay for all stages of syphilis is:
    A. FTA-Abs
    B. MTA-TP
    C. RPR
    D. VDRL

17. The following test is the most accurate indication of the presence of inflammation:
    A. ESR
    B. CRP
    C. ANA
    D. RPR

18. Laboratory assays are performed on a patient with suspected thyroid disease. Listed below are the results:

Test	Result
Anti-TPO (TPO Ab)	elevated
TSHR Ab	elevated
TSI	elevated
Anti-Tg (Tg AB)	normal

These results are consistent with which of the following?
A. Hashimoto's thyroiditis
B. Graves disease
C. Thyroid cancer
D. Hypothyroidism

ANSWERS AND RATIONALE

1. A

To properly assess an infection as current, there must be a 4-fold rise in the antibody titer. If the first titer was 320, subsequent tests should give a titer of at least 1280 if infection is current.

2. A

Active immunity occurs after patient is presented with an antigen *(infectious organism or immunization)* and the patient produces antibodies.

Option B is incorrect since passive immunity occurs when antibody is produced by another person or animal and the antibodies are transferred to the patient giving the patient temporary immunity. *(The patient does not produce the antibodies.)* Option C, adoptive immunity, occurs when immunocompetent cells are transferred to the patient. Option D, natural immunity, refers to the nonspecific mechanisms involved in fighting infection. It does not result in antibody formation.

3. D

The presence of antibody to Epstein Barr Virus nuclear antigen indicates a past infection. Anti-VCA is probably IgG rather than IgM.

4. B

Speckled patterns are confirmed using double immunodiffusion, radial immunodiffusion, indirect immunofluorescence or enzyme immunoassay to test for antibodies to specific saline extractable nuclear antigens *(anti-Sm, anti-ds DNA, anti-Scl-70, etc.)* Option A is incorrect because repeating the test is unnecessary. Option C is incorrect since mitotic cells are not positive in speckled patterns. Option D is incorrect because *Crithidia* substrate is used to confirm a homogeneous pattern.

5. C

In multiple sclerosis, IgG oligoclonal bands are often seen in CSF. Option A is incorrect because 40 - 70% of patients manifest antibodies against elements of the adrenal cortex and adrenal cell surfaces. Option B is incorrect since patients with myasthenia gravis demonstrate acetylcholine receptor blocking antibodies. *(IgG, C3 and C9 can be demonstrated at the neuromuscular junctions).* Option D is incorrect because multiple myeloma *(plasma cell myeloma)* is characterized by neoplastic proliferation of a single clone *(monoclonal)* of plasma cells that produce a specific type of immunoglobulin *(usually IgG).*

6. C

When HBeAg is present, the patient is considered highly infectious. Option A is incorrect because all antigen and antibody results would be negative during the incubation period. Option B is incorrect because anti-HBc would be negative in a very early infection. Option D is incorrect because an immune individual would be positive for anti-HBsAg.

7. D

The patient's titer is not definitive. He/she could have antibodies from a past infection or from immunization. To determine if the infection is current, the test should be repeated in about two weeks with the results demonstrating a titer of at least 80 *(4-fold increase).*

8. A

VDRL is the test of choice for testing CSF.

9. C

Due to reduced numbers of T cells in acquired immunodeficiency disease, there is a decrease in the T helper / T regulator ratio.

10. C

Proliferation of a monoclonal antibody in multiple myeloma is usually of the IgG class.

11. D

Radioallergosorbent testing (*RAST*) is used to measure IgE antibodies to specific allergens. Specific allergen assays may also be performed using ELISA techniques.

12. B

FTA-ABS *(fluorescent treponemal antibody absorption)* is used to confirm positive screening tests (*RPR, VDRL*) for syphilis. Many diseases other than syphilis such as SLE, infectious mononucleosis, hepatitis, and malaria give false positive results for screening tests. Since the FTA-ABS is negative, this is probably a biologic false positive.

13. B

The initial tube indicates that prozone has occurred. Therefore, the last tube showing agglutination is 1:160. The titer of 160 is the reciprocal of the dilution of the last tube displaying agglutination.

14. D

If an ANA pattern is speckled, follow up testing is performed for antibodies directed against extractable nuclear antigens to confirm the test and aid in determining the autoimmune disease present. Anti-SSA and anti-SSB are often positive in patients with Sjogrens syndrome. Patients with anti-Sm often have SLE. Anti-Scl 70 is associated with scleroderma and anti-RANA is associated with rheumatoid arthritis.

15. B

CA 19-9 is often elevated in patients with pancreatic cancer. CEA is elevated in many cancers including breast cancer and colon cancer. CA125 is a marker used in ovarian cancer. CA15-3 is used in patients with breast cancer.

16. A

FTA-Abs remains positive after treatment. Non-treponemal tests are not positive after the patient is treated.

17. B

CRP is the most sensitive measure of the rise and fall of inflammation. Concentrations of CRP increase within 4-6 hours of the onset of inflammation and decrease rapidly when inflammation subsides. ESR indicates inflammation but does not rapidly reflect changes in inflammatory status.

18. B

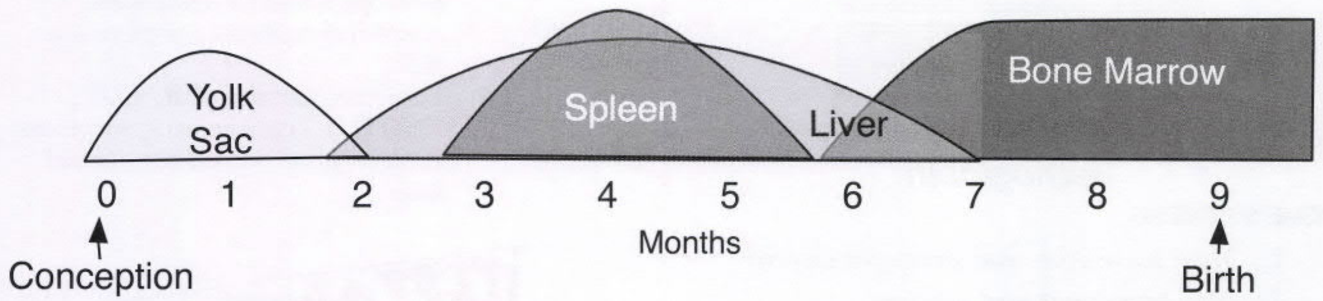
TPO Ab, TSHR Ab and TSI are elevated in Graves Disease and hyperthyroidism. Tg Ab may also be elevated in thyroid cancer.

HEMATOLOGY

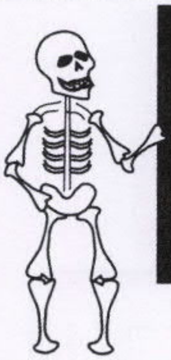
by Angela B. Foley

Hematopoiesis

Prenatal Hematopoiesis

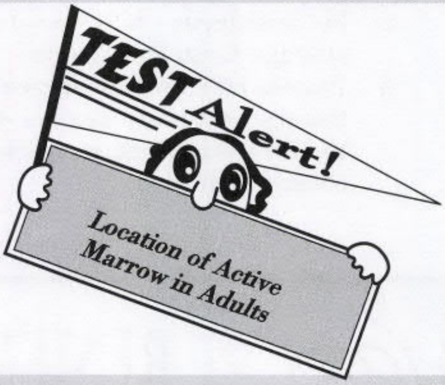


Adult Hematopoiesis



SKULL
STERNUM
PELVIS
RIBS
VERTEBRAE

Flat Bones: Active Adult Hemopoietic Tissue (colony forming units in red marrow)


TEST Alert!
Location of Active Marrow in Adults

Blood Cell Maturation

HEMATOPOIETIC STEM CELL					
	CFU - GEMM				CLP
COMMITTED PROGENITORS	**CFU-E**	**CFU-GM [EO, BASO]**		**CFU-MK**	**CFU-TNK & CFU-B**
GROWTH FACTORS	**GM-CSF, IL-3, EPO**	**GM-CSF, IL-3,5**		**GM-CSF, IL-3,6,11, TPO**	**IL-1,2,4,5,6,7,12,15**
Bone Marrow (BM)	Rubriblast	Myeloblast	Monoblast	Megakaryoblast	Lymphoblast
	Prorubricyte	Promyelocyte	Promonocyte	Promegakaryocyte	Prolymphocyte
	Rubricyte	Myelocyte			
	Metarubricyte	Metamyelocyte		Megakaryocyte	
Peripheral Blood	Reticulocyte	Band Neutrophil			
	Erythrocyte	Segmented Neutrophil	Monocyte	Platelet (thrombocyte)	Lymphocyte
		Basophil Eosinophil	Macrophage (tissue)		NK Cell T Cell B Cell
					Plasma Cell

EPO = Erythropoietin
CSF = Colony Stimulating Factor
TPO = Thrombopoietin
GEMM = Granulocyte, Erythrocyte, Megakaryocyte, Monocyte
CLP = Committed Lymphoid Progenitor
IL = Interleukin

GM = Granulocyte, monocyte
CFU = Colony Forming Unit
MK = Megakaryocyte
L = Lymphocyte
NK = Nautural Killer

Action of EPO and TPO

ID Mature and Immature Cells from Graphic Images

Hemoglobin

HEME SYNTHESIS

1. Must have iron and protoporphyrin
2. Iron transport and storage
   a. Transferrin - Fe transport protein
   b. Ferritin - major Fe storage form
   c. Hemosiderin - H_2O insoluble Fe storage form (long-term)
   d. Excess iron will be stored in tissues and body organs ➔ can lead to hemosiderosis, hemochromatosis (organ damage)
3. Protoporphyrin Synthesis
   a. Precursors *(see memory tool below)*
   b. Porphyrias - Enzyme deficiencies cause build-up of heme precursors – red or port wine colored urine
      - ❖ *Early precursors (DELTA ala or PORphobilinogen) – neuropsychiatric symptoms, i.e. acute intermittent porphyria or AIP*
      - ❖ *Later precursors (UR, COP, PROTO) – cutaneous sysmptoms such as photosensitivity, facial hair*

Conditions Associated with Increased Storage of Iron

Fe Transport Protein
Fe Storage Forms

REMEMBER!

Heme Precursors

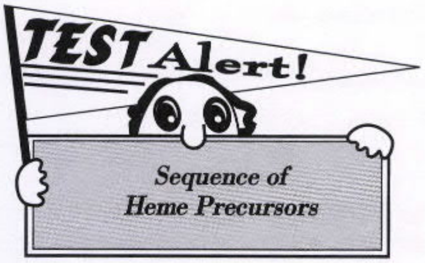
DELTA
DINER
Y'ALL
ARE
HERE
MS
AL
CUP O' HEME

While in the DELTA, POUR YOUR COP, PRONTO, a cup of HEME.

DELTA-aminolevulinic acid
↓
PORphobilinogen
↓
URoporphyrinogen
↓
COProporphyrinogen
↓
PROTOporphyrin
↓
HEME + globin 4 hemoglobin

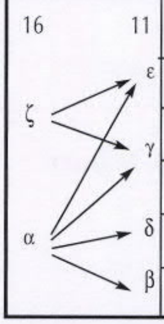
PORPHYRIAS:

Exessive formation of porphyrins occurs if any enzymatic step in heme synthesis is blocked.



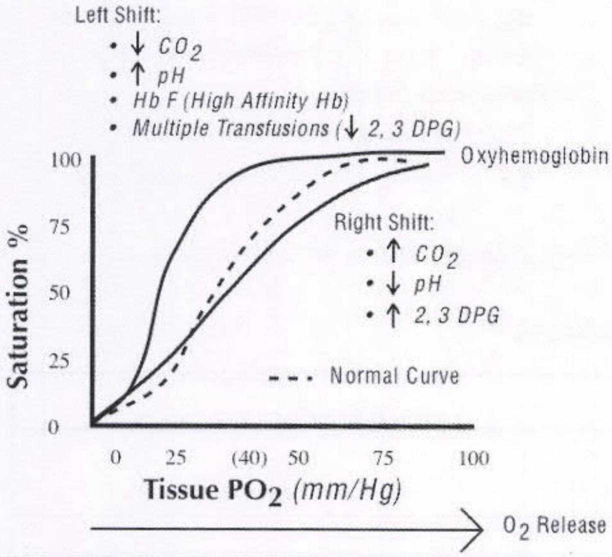
GLOBIN SYNTHESIS

HEMOGLOBIN TYPES IN NEWBORN AND ADULT

CHROMOSOME	GLOBIN CHAINS	HGB	NEWBORN %	ADULT %
	$\zeta_2\,\varepsilon_2$	Gower I	0% (Embryonic)	0% (Embryonic)
	$\alpha_2\,\varepsilon_2$	Gower II		
	$\zeta_2\,\gamma_2$	Portland		
	$\alpha_2\,\gamma_2$	**Hgb F**	60-90%	1%
	$\alpha_2\,\delta_2$	**Hgb A_2**	<2%	2%
	$\alpha_2\,\beta_2$	**Hgb A**	10-40%	97%

HEMOGLOBIN-OXYGEN DISSOCIATION CURVE

1. Shift to left - O_2 NOT released to tissue adequately
2. Shift to right - O_2 released to tissue more easily

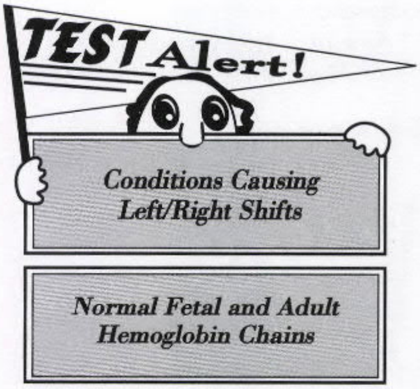


REMEMBER!

Left/Right Shifts

Shift Right - Won't Hold Tight

Shift Left - No! Won't Let Go


TEST Alert!
Conditions Causing Left/Right Shifts
Normal Fetal and Adult Hemoglobin Chains

Basic Laboratory Procedures

ANTICOAGULANTS

1. EDTA (*ethylenediamine-tetra-acetate*) - chelates Ca^{++}
2. Heparin - anti-thrombin agent

HEMOGLOBIN

1. Principle - conversion of hemoglobin to cyanmethemoglobin by potassium cyanide and potassium ferricyanide
2. Sources of error
   a. Lipemia / icterus
   b. High white count
   c. Resisting hemoglobins (*i.e. SS, CC*)
   d. Excess hemolysis
3. Reference range - male: 16 ± 2 g/dl
   female: 14 ± 2 g/dl

HEMOGLOBIN VARIANTS

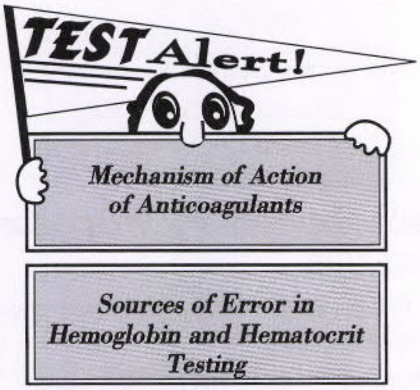
HEMOGLOBIN	COMMENTS
Methemoglobin	$Fe2^+$ Oxidized to $Fe3^+$, Brown, Cannot Bind O_2
Carboxyhemoglobin	↑in Carbon Monoxide Poisoning & Smokers, Cherry-Red
Sulfhemoglobin	Only hemoglobin not measured by cyanmethemoglobin method

HEMATOCRIT

1. Measures packed cell volume in percent
2. Microhematocrit method
   a. Can use fingerstick, EDTA or heparinized sample
   b. Sources of error
      - ❖ *Failure to seal tube adequately*
      - ❖ *Incorrect reading due to uneven clay plug*
      - ❖ *Inappropriate centrifuge time / speed*
      - ❖ *Excess EDTA resulting in RBC shrinkage*
3. Automated method
   a. Calculated from MCV and RBC

b. Sources of error
- ❖ *Cold Agglutinins*
- ❖ *High WBC Count*

4. Reference range - male: 47 ± 5%
female: 42 ± 5%

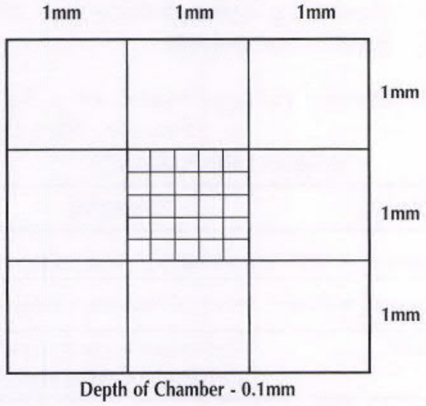


Mechanism of Action of Anticoagulants

Sources of Error in Hemoglobin and Hematocrit Testing

MANUAL CELL COUNTS

1. WBC/Platelet Count
   - a. Diluent - 1.98 ml of 1% Ammonium Oxalate
   - b. 0.02 ml or 20µl pipet
   - c. 1:100 dilution *(0.02 ml in 2.00 ml)*
2. Hemacytometer *(Neubauer)*



Depth of Chamber - 0.1mm

3. Calculations

Formula:

$$\# \text{ cells counted} \times \frac{1}{\text{Tot vol*}} \times \text{dilution factor}$$

NOTE:
Volume (of 1 square) = length x width x depth
*Total volume = volume x # of squares counted

a. WBC count
- ❖ *Count all 9 squares*
- ❖ *Total volume counted = 1mm x 1mm x 0.1mm x 9 = 0.9mm^3*
- ❖ *Reference range - 5,000 - 10,000/ul*

b. Platelet count
- ❖ *Count all 25 squares in center large square*
- ❖ *Total volume counted = 1mm x 1mm x 0.1mm^3 = 0.1mm^3*
- ❖ *Reference range - 150,000 - 400,000/ul*

❖ ***WBC example***

WBC's counted = 60

$$60 \times \frac{1}{.9} \times 100 = 6{,}667/\text{mm}^3 \text{ or } \mu\text{L}$$

NOTE: *You can eliminate the 1/.9 (volume factor) by multiplying 10% of cells counted and adding this value to the number of cells counted.*
Example:
60 cells counted x 10% = 60 + 6 = 66
Now: 66 x 100 = 6,600/mm^3 or µL

❖ ***Platelet example***

Platelets counted = 150

$$150 \times \frac{1}{.1} \times 100 = 150{,}000/\text{mm}^3 \text{ or } \mu\text{L}$$

NOTE: *Multiply number of platelets counted by 1000.*

Red Cell Indices

INDICES	FORMULA	REF. RANGE	- - - - - - - - INDICATION - - - - - - -	
MCV*	$\frac{HCT}{RBC} \times 10$	80-100fl (μ^3)	<80 >100	Microcytes Macrocytes
MCH	$\frac{HB}{RBC} \times 10$	28-32pg (µµg)		Varies with Hemoglobin Content and Cell Size
MCHC	$\frac{HB}{HCT} \times 100$	32-36% (g/dl)	<32 >36	Hypochromic Cells, Icterus Spherocytes, Icterus, Lipemia, Hb SS, CC
RDW-CV	$\frac{\text{SD of MCV} \times 100}{\text{Mean MCV}}$	11.5-14.5%	>14.5	Anisocytosis
RDW-SD	Width of peak at 20% height	39-47 fL	>47 fL	Anisocytosis

* MCV is directly measured by automated methods; Hct is calculated from MCV and RBC count.

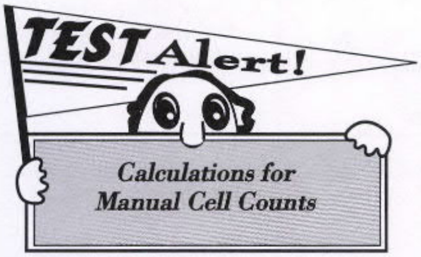
Peripheral Blood Cells and Their Function

CELL	REFERENCE RANGE	FUNCTION
Red Cell	Females 3.8 - 5.2 x 10^6/µl Males 4.5 - 6.1 x 10^6/µl	O_2 Transport to Tissue and CO_2 Removal from Tissue
Neutrophil	Relative 45-70% Absolute 2250-7000/µl	Injest and Kill Bacteria
Lymphocyte	Relative 20-40% Absolute 1000-4000/µl	Humoral and Cell Mediated Immunity
Monocyte	Relative 3-10% Absolute 150-1000/µl	Injest and Kill Bacteria, Digest Debris, Initiate and Regulate Adaptive Immune Response
Basophil	Relative 0-2% Absolute 0-200/µl	Inflammatory Response Mediator
Eosinophil	Relative 0-3% Absolute 0-300/µl	Allergic Response Regulator
Platelet	Absolute 150,000-400,000/µl	Clotting

BLOOD SMEAR EXAMINATION

1. Wright stain
   a. Polychrome (*Romanowsky)* stain - phosphate buffer (pH 6.4), eosin, methylene blue and methanol (fixative)
   b. Sources of error
      - ❖ *Stain too blue (pH of buffer or stain too basic, prolonged staining)*
      - ❖ *Stain too red (pH of buffer or stain too acid, prolonged washing)*

2. Leukocyte differential
   a. Normal Relative (%) values - see chart above
   b. Absolute values
      ❖ *Calculation*
      Relative value (%) x Total WBC count
      ❖ *Example*
      Total WBC count = 6000/uL
      % Neutrophils from diff = 60%
      Absolute neutrophil count =
      0.6 x 6000/uL = 3,600/uL



Calculations for Manual Cell Counts

Wright Stain Components

Sources of Error in Staining Smears

c. Significance of increased leukocytes

CELL	INCREASED IN
Neutrophil	Bacterial Infections
Lymphocyte	Viral Infections
Monocyte	TB, Syphilis, Malignancies
Eosinophil	Allergies, Parasites
Basophil	Immediate Hypersensitivity

d. White cell morphology

MORPHOLOGY	ASSOCIATED WITH
Hypersegmented Neutrophil	Megaloblastic Anemia (*B12 and folate deficiency*)
Hyposegmented Neutrophil	Pelger Huet , Pseudo-Pelger Huet (*AML*, AIDS*)
Toxic Granulation and Vacuoles	Bacterial Infections, Burns, Chemotherapy
Döhle Bodies (RNA)	Bacterial Infections, Burns, May-Hegglin
Variant Lymphs (increased size and basophilia)	Infectious Mono, Other Viral Infections

* Acute Myeloid Leukemia

3. **Platelet estimate**
   a. 8-20/oil immersion field- adequate
   b. If platelets seem low, check feather edge of slide for platelet clumping, check for satelitism (*EDTA related-redraw in Na citrate)*
   c. Check platelet size
      - ❖ *Large - Bernard-Soulier, May- Hegglin, myeloproliferative disorders, stress platelets*

4. **Nucleated red cells**
   a. Must correct white cell count (*if included in count)*
   b. Formula

$$\frac{\text{wbc count x 100}}{\text{100 + \# nrbcs}} = \text{corrected WBC count}$$

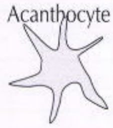
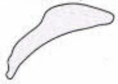
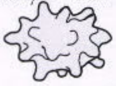
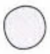
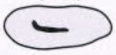
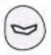
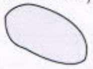
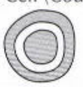
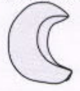
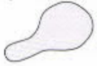
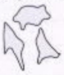
5. **Red cell morphology**
   a. Size
      - ❖ *Normocytic (MCV of 80-100μ^3 or fL)*
      - ❖ *Microcytic (MCV < 80)*
      - ❖ *Macrocytic (MCV > 100)*
      - ❖ *Anisocytosis - variation in size (RDW> 14.5)*
   b. Color
      - ❖ *Normochromic (MCHC of 32-36 g/dL)*
      - ❖ *Hypochromic (MCHC < 32)*
      - ❖ *Hyperchromic (MCHC > 36)*
      - ❖ *Polychromasia - blue color in rbc (if stained with new methylene blue, these would be reticulocytes)*
   c. Shape - Bi-concave disk
      - ❖ *Poikilocytosis - variation in shape (see chart below)*
   d. Inclusions (*see chart page 59*)
   e. Crystals
      - ❖ *Hemoglobin C - bar-shaped*
      - ❖ *Hemoglobin SC - "hand in glove" or "Washington monument"*

WBC Reference Ranges, Relative vs Absolute Values, Cell Functions

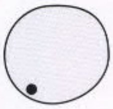
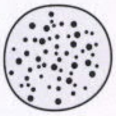
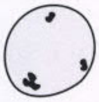
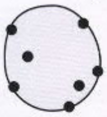
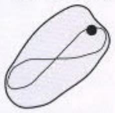
Correlate Conditions with Increased WBCs and Abnormal Morphology

Action Taken if Low Platelets Seen on Smear

Abnormal Red Cell Shapes

SHAPE	SEEN IN:	SHAPE	SEEN IN:
Acanthocyte 	Abetalipoproteinemia, Severe Liver Disease	Sickle Cell (Drepanocyte) 	Hb SS
Echinocyte "Burr" Cell 	Uremia, Artifact *(Alkaline Glass Effect)*	Spherocyte 	Hereditary Spherocytosis, (↑ *MCHC*), ABO HDN, and Other Hemolytic Processes
Elliptocyte 	Hereditary Elliptocytosis, Iron Deficiency, Thalassemia	Stomatocyte (Mouth Cell) 	Hereditary Stomatocytosis Liver Disease
Macroovalocyte 	Megaloblastic Anemia	Target Cell (Codocyte) 	Liver Disease, Hb C, Thalassemias, and Other Hemoglobinopathies
Helmet (Keratocyte) 	Hemolytic Processes	Teardrop Cell (Dacryocyte) 	Extramedullary Hematopoiesis, Thalassemias, Pernicious Anemia
Schistocyte (RBC Fragment) 	DIC and Hemolytic Processes	*NOTE:*	HDN - Hemolytic Disease of the Newborn DIC - Disseminated Intravascular Coagulation)

Red Cell Inclusions

INCLUSION	COMPOSED OF:	STAIN	INDICATIONS
Howell-Jolly Body 	DNA	Wright New Methylene Blue	Disturbed Erythropoiesis Hemolytic Anemias Megaloblastic Anemia Post-Splenectomy
Basophilic Stippling 	RNA	Wright New Methylene Blue	Thalassemia Lead Poisoning
Pappenheimer Bodies Siderotic Granules *(siderocyte)* 	Iron	Wright Confirm with Prussian Blue	Sideroblastic Anemia Hemoglobinopathies
Heinz Body 	Denatured Precipitated Hemoglobin	Supravital Stain such as Brilliant Cresyl Blue or New Methylene Blue **(NOT seen with Wright stain)**	G6PD Deficiency Thalassemia Unstable Hemoglobins
Cabot Ring 	Remnants of Mitotic Spindle	Wright	Megaloblastic Anemia
Parasites	Malaria Babesia Trypanosomes	Wright	Parasitic Infection

Correlate Abnormal RBC Shapes with Conditions

Correlate Abnormal RBC Inclusions with Conditions

RULE OF THREE

Correlation of Hb, Hct and RBC

Hb x 3 = Hct ± 3%

RBC *(in millions)* x 3 = Hb ± *0.5*

If "Rule of Three" doesn't fit, consider:

1. *Clotted sample*
2. *Cold agglutinin (warm sample and rerun)*
3. *Lipemic, icteric, or grossly hemolyzed sample*
4. *Resisting hgb (SS, CC)*
5. *Abnormal red cells*

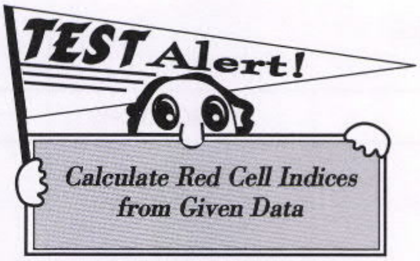
NOTE:

☞ *↑ MCV, ↑ MCHC and ↓ red cell count are associated with cold agglutinin disease; warm sample and rerun*

REMEMBER!

HEME NORMALS

	♀	♂
Hb	14 ± 2 g/dL	16 ± 2 g/dL
Hct	42 ± 5%	47 ± 5%
RBC	$4.5 \pm 0.7\times10^6/\mu L$	$5.3 \pm 0.8\times10^6/\mu L$
WBC	5,000 - 10,000/μL	
Platelet	150,000 - 400,000/μL	



Calculate Red Cell Indices from Given Data

Obtain Accurate Results on Specimen with Cold Agglutinin

ERYTHROCYTE SEDIMENTATION RATE

1. Principle - measures rate of fall of red cells through plasma
2. Reference ranges
   a. Female: 0-20 mm
   b. Male: 0-15 mm
3. Clinical correleation
   a. ↑ seen in presence of increased plasma proteins, primarily fibrinogen and globulin *(inflammation/infection)*
4. Sources of error
   a. Increase - tilting tube, standing too long, ↑ temperature, excess EDTA
   b. Decrease - QNS specimen, ↓ temperature, excess EDTA

RETICULOCYTE COUNT

1. Uses a supra vital stain which stains red cells in living state
   a. New methylene blue
   b. Brilliant cresyl blue
2. Monitors erythropoiesis
3. Calculations
   a. % reticulocytes =

$$\frac{\text{\# retics in 1000 RBCs}}{10}$$

   b. Absolute retic = # RBCs x % retics
   c. Corrected retic count =

$$\frac{\text{\% retics x patient hct}}{45}$$

4. Reference ranges - 0.5 - 1.5%
5. RPI (Reticulocyte Production Index =

$$\frac{\text{Corrected retic count}}{\text{maturation time (usually use 2)}}$$

   a. > 2 - adequate bone marrow (BM) response to anemia
   b. < 2 - inadequate BM response to anemia
6. ↑ in hemolytic anemias, post-acute blood loss, following therapy *(e.g., iron, folate, B12)*

Reticulocyte Calculation Examples

RBC = 3.0 x 10^6/µL; HCT = 25%; 36 retics counted in 1000 RBCs

❖ *Retic count example:*

$$\frac{36}{10} = 3.6\%$$

❖ *Absolute retic count example:*

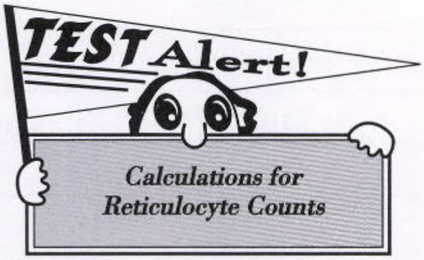
$3.0 \times 10^6/\mu L \times .036 = 108 \times 10^3/\mu L$ or $.108 \times 10^6/\mu L$

❖ *Corrected retic count example:*

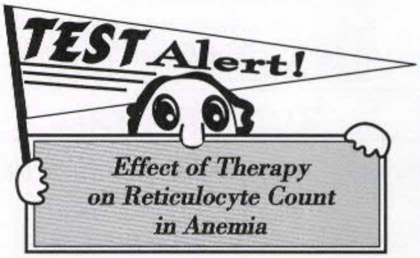
$$\frac{3.6 \times 25}{45} = 2.0\%$$

❖ *RPI example:*

$$\frac{2.0}{2} = 1.0$$



Calculations for Reticulocyte Counts



Effect of Therapy on Reticulocyte Count in Anemia

Relative vs. Absolute Values

Special Hematology

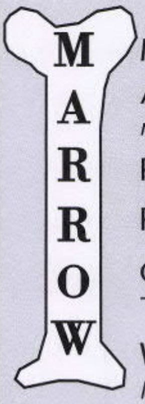
BONE MARROW PREPS

1. Reference ranges
   a. Megakaryocytes - 5/lpf
   b. Myeloid: erythroid ratio - 3:1 - 4:1

2. Clinical correlations
   a. "Dry" tap - aplastic anemia, myelofibrosis
   b. ↓M:E ratio - erythroid hyperplasia, hemolytic anemia, erythroleukemia
   c. ↑M:E ratio - myeloid hyperplasia, myeloid leukemias

REMEMBER!

Bone Marrow



M:E Ratio 3:1 - 4:1

Aspirate - *sternum & hip (iliac crest) most common - for morphology review*

Romanowsky Stains

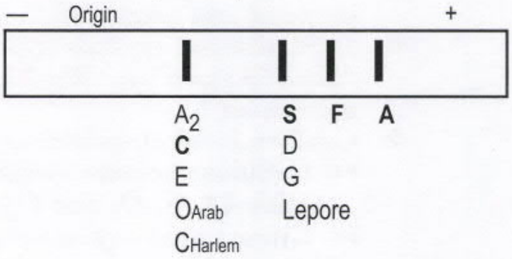
Ratio of Cells:Fat *(50% cells:50% fat)*

Overall Evaluation of Cellularity *- use biopsy*

WBC Differential *(determine type of leukemia)*

HEMOGLOBIN ELECTROPHORESIS

1. Cellulose acetate, pH 8.6; from cathode to anode *(- to +)*

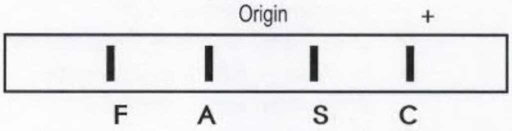


REMEMBER!

Hemoglobin Migration (pH 8.6)

C *(crawl)*, **S** *(slow)*, **F** *(fast), and* **A** *(accelerate)*

2. Citrate Agar, pH 6.2



(Most other hemoglobins migrate with A at pH 6.2)

REMEMBER!

What Migrates with A_2 & S (pH 8.6)

A_2, CE Of C lubs (Harlem)

Sad Dog Gets Loved

Red Cell Disease States

HEMOGLOBINOPATHIES

1. Structural mutations (most common involve β-chain)

HEMOGLOBIN	COMMENTS
S	Valine for Glutamic Acid *(6th Position, Beta Chain)*
C	Lysine for Glutamic Acid *(6th Position, Beta Chain)*
D	East Indian Individuals, Migrates with HbS at 8.6
E	Southeast Asian Individuals, Migrates with Hb C and A2 at 8.6 *(hypochromic, microcytic)*

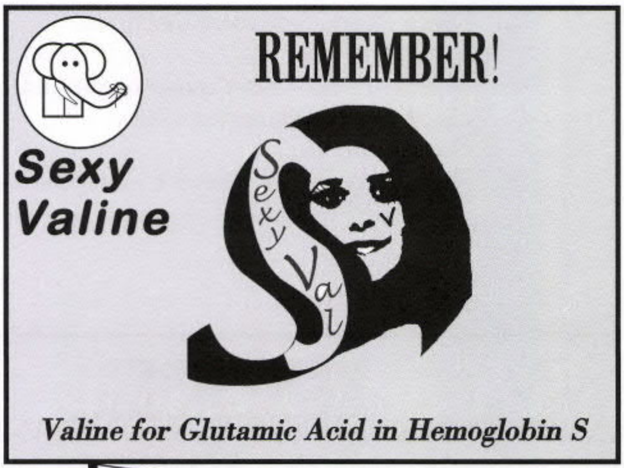
a. Hemoglobin S
- *Heterozygous - asymptomatic (sickle cell trait)*
- *Homozygous - severe chronic hemolytic anemia with many complications (sickle cell disease)*
- *See sickle cells on Wright (not in trait)*
- *Sickle Dex - reducing agent (Na dithionite) causes Hb S to precipitate producing turbidity*
- *Confirmed by electrophoresis*
  - ☞ Cellulose acetate - pH 8.6 *(migrates with D, G, Lepore)*
  - ☞ Citrate agar - pH 6.2 *(S separates from others)*

b. Hemoglobin C
- ❖ *Heterozygous - asymptomatic*
- ❖ *Homozygous - mild chronic anemia*
- ❖ *May see Hb C crystals (bar-shaped), and target cells*
- ❖ *In SC disease, crystals appear as "hand in glove" or "Washington monument"*
- ❖ *Confirm by electrophoresis*
  - ☞ Cellulose acetate - *migrates with A2, E, O, and C_{Harlem}*
  - ☞ Citrate agar - *(C separates from others)*

2. Decreased production of α or β chains

a. β thalassemia - ↓ or absent production of β-chains
- ❖ *Microcytic, hypochromic anemia*
- ❖ *↑ Hb A_2 and F, ↓ or absent A*

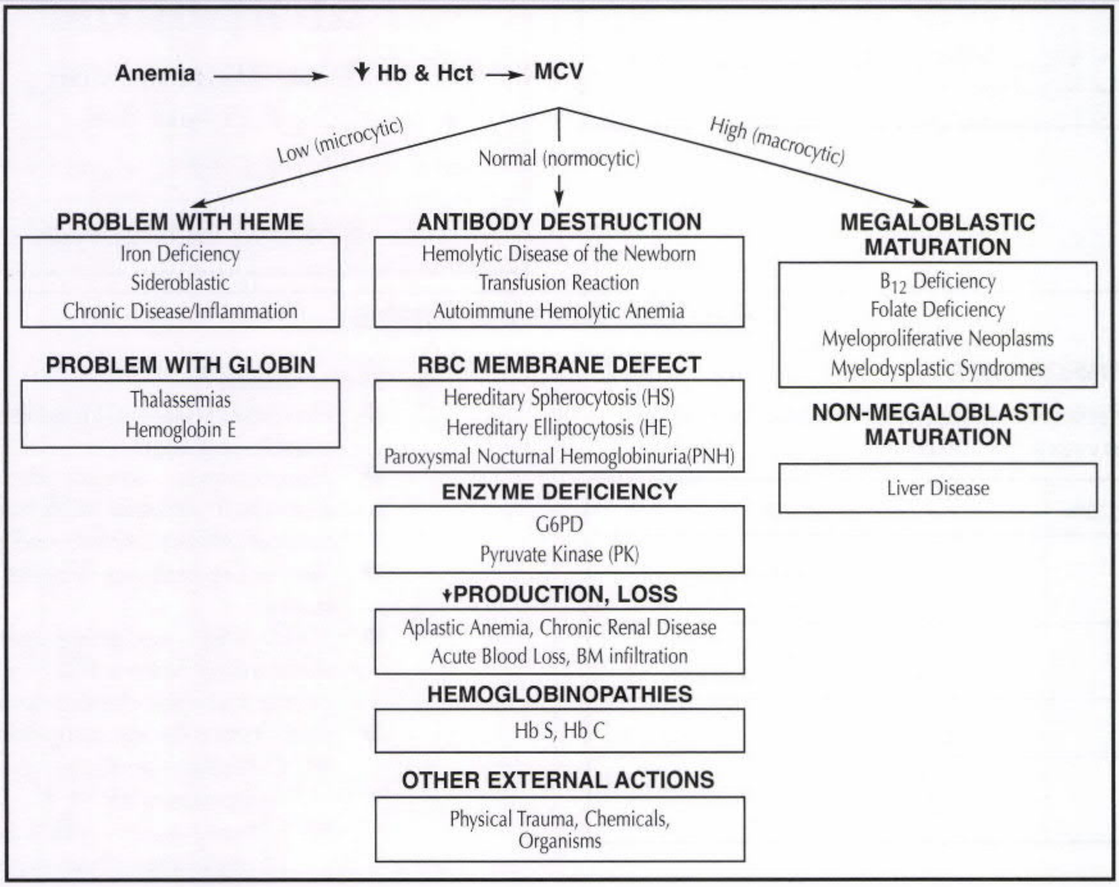
b. α thalassemia - ↓ production of α-chains
- ❖ *1 deleted α gene (- α/αα) - Silent carrier; normal CBC*
- ❖ *2 deleted α genes (- α/-α) or (- -/αα) - Mild microcytic, hypochromic anemia*
- ❖ *3 deleted α genes (- - /-α) - Hemoglobin H disease*
  - ☞ Chronic hemolytic disease
  - ☞ Hb H (β_4)
  - ☞ Hb Bart's (γ_4) present at birth
  - ☞ Hb H inclusions *(heinz bodies)*
- ❖ *4 deleted α genes (- -/- -)*
  - ☞ *Hydrops fetalis*
  - ☞ Nonviable fetus


REMEMBER!
Sexy Valine
Sexy Val

Valine for Glutamic Acid in Hemoglobin S

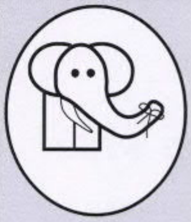
Hemoglobin Variants and Electrophoretic Migrations

Classification of Anemias


Anemia
↓ Hb & Hct
MCV
Low (microcytic)
Normal (normocytic)
High (macrocytic)
PROBLEM WITH HEME
Iron Deficiency
Sideroblastic
Chronic Disease/Inflammation
PROBLEM WITH GLOBIN
Thalassemias
Hemoglobin E
ANTIBODY DESTRUCTION
Hemolytic Disease of the Newborn
Transfusion Reaction
Autoimmune Hemolytic Anemia
RBC MEMBRANE DEFECT
Hereditary Spherocytosis (HS)
Hereditary Elliptocytosis (HE)
Paroxysmal Nocturnal Hemoglobinuria(PNH)
ENZYME DEFICIENCY
G6PD
Pyruvate Kinase (PK)
↓PRODUCTION, LOSS
Aplastic Anemia, Chronic Renal Disease
Acute Blood Loss, BM infiltration
HEMOGLOBINOPATHIES
Hb S, Hb C
OTHER EXTERNAL ACTIONS
Physical Trauma, Chemicals, Organisms
MEGALOBLASTIC MATURATION
B12 Deficiency
Folate Deficiency
Myeloproliferative Neoplasms
Myelodysplastic Syndromes
NON-MEGALOBLASTIC MATURATION
Liver Disease

Anemia Types and Key Lab Findings

ANEMIA	LAB FINDINGS
MICROCYTIC/HYPOCHROMIC	
• Iron Deficiency	• ↓ Serum Ferritin, ↓Serum Fe, ↑TIBC, ↓% Saturation
• Chronic Disease / Inflammation	• N-↑ Serum Ferritin, ↓Serum Fe, ↓TIBC
• Lead Poisoning	• Basophilic Stippling, ↑Blood Pb, ↑FEP
• Thalassemia Trait	• N Serum Fe, N TIBC, ↑A2, ↑F
MACROCYTIC	
• B12 Deficiency ☞ Pernicious Anemia (PA)	• ↓B12, ↓Retics, Pancytopenia, Oval Macrocytes, Hypersegmented Polys, Howell Jolly (HJ) Bodies ☞ Anti-IF positive (Intrinsic Factor), ↑MMA (methylmalonic acid), ↑Homocysteine,
☞ Malabsorption/ Dietary	☞ ↓B12, Anti-IF negative
• Folate Deficiency	• ↓Folate levels, Anti-IF negative, ↓Retics, Oval Macrocytes, Hypersegmented Polys
• Liver Disease / Alcoholism	• ↑Liver Enzymes, Target Cells, Round Macrocytes
NORMOCYTIC/NORMOCHROMIC	
• Antibody Mediated	• ↑Bilirubin, ↓Haptoglobin, DAT+
☞ PCH	☞ Donath Landsteiner Ab (Anti P specificity)
☞ Cold Agglutinin Disease	☞ IgM Ab (Anti I specificity), Cold Agglutinin Titer+
☞ Warm Autoimmune Hemolytic Anemia	☞ IgG Ab
• Membrane Defect	
☞ Hereditary Spherocytosis	☞ Spherocytes, ↑MCHC, Abnormal Spectrin
☞ Hereditary Elliptocytosis	☞ Elliptocytes (>15% to 100%), Abnormal Spectrin
☞ PNH	☞ CD55–, CD59–, FLAER Test positive
• Enzyme Deficiency	
☞ G6PD	☞ ↓G6PD, Heinz Bodies
☞ Pyruvate Kinase (PK)	☞ ↓PK, No Heinz Bodies
• Decreased Production / Loss	
- Aplastic Anemia	☞ "Dry Tap" Bone Marrow (BM), Hypocellular BM, ↓Retics, Pancytopenia
- Acute Blood Loss	☞ Normal BM, ↑Retics
- Chronic Renal Disease	☞ ↓EPO
• Hemoglobin Defects	• Definitive Poikylocytes on Smear (HbC crystals, Sickle Cells, SC crystals, etc.), Hb Electrophoresis

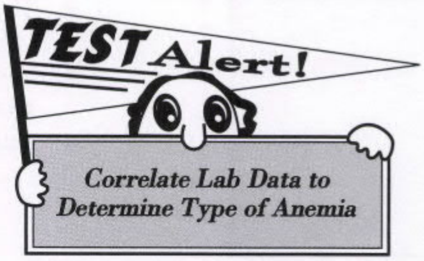


REMEMBER!

Trust in yourself.

Your perceptions are often far more accurate than you are willing to believe

— *Claudia Black*



Correlate Lab Data to Determine Type of Anemia

Special Tests

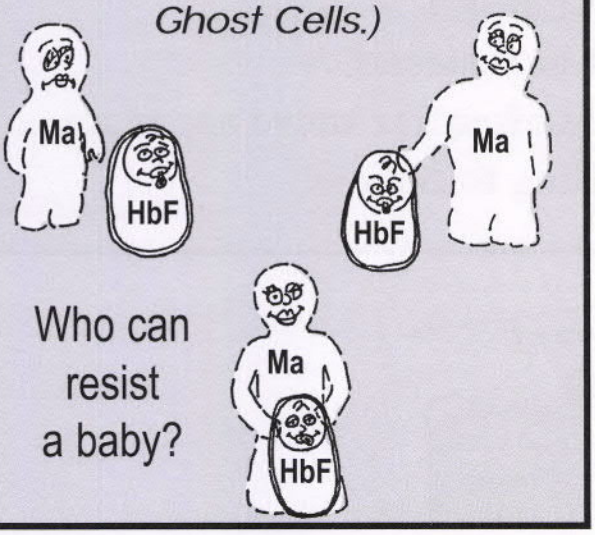
TEST	MEASURES	INDICATIONS	COMMENTS
FLAER Test (Fluorescent Aerolysin)	Absence of GPI Anchor Proteins for CD 55 and CD59	PNH	More specific and sensitive
Flow Cytometry	Deficiency of CD 55 and CD 59 on RBCs and granulocytes	PNH	Less sensitive than FLAER test
Heinz Body Prep (supravital stain)	Effect of Oxidizing Agent on Hemoglobin (Precipitated globin chains)	G6PD Deficiency Unstable Hemoglobins HbH	Formation Triggered by Oxidants such as Anti-Malarial Drugs, Fava Beans & Sulphur Drugs
Sickle Cell Screen	Reduced Solubility of Deoxygenated Hemoglobin S	HbS	Reducing Agent: Na Dithionate
Kleihauer-Betke Acid Elution	Resistance of Fetal Hemoglobin to Acid Elution	Fetal-Maternal Hemorrhage; Hereditary Persistence of Fetal Hemoglobin	Cells with ↑ HbF Stain Pink; Normal Adult Cells → Ghost Cells
Hemoglobin Electrophoresis	Migration of Various Hemoglobins	Suspected Hemoglobinopathies	May be Performed at Various pHs
Cold Agglutinin Titer	Presence of Cold Autoantibody	Cold Autoimmune Hemolytic Anemia	IgM Ab, Anti-I Specificity
Donath Landsteiner Test	Presence of Biphasic DL Antibody	Paroxysmal Cold Hemoglobinuria	IgG Ab, Anti-P specificity

GPI- Glycosylphosphatidylinositol
FLAER- Fluorescent Aerolysin

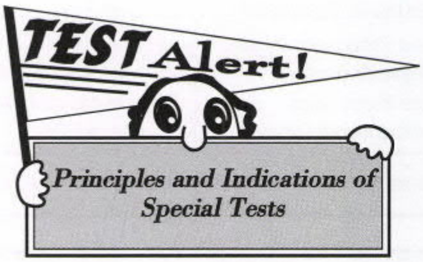
REMEMBER!

Kleiheur-Betke

Fetal Hb is Resistant to Acid Elution *(Adult Cells Appear as Ghost Cells.)*



Who can resist a baby?



Principles and Indications of Special Tests

ERYTHROCYTOSES

1. **↑number of red cells *(↑Hb,↑Hct)***
2. **Relative *(↓plasma vol)* - burns, dehydration, Gaisbock syndrome**
3. **Absolute *(↑ red cell mass)***
   - a. **Primary - ↓EPO, normal O_2 saturation *(polycythemia vera)***
   - b. **Secondary - ↑EPO, ↓O_2 saturation *(chronic obstructive lung disease, high O_2 affinity Hb)***

White Cell Disease States

HEREDITARY CONDITIONS

CONDITION)	CHARACTERISTICS	COMMENTS
Alder-Reilly	Large Azurophilic Granules	↑Mucopolysaccharides (Hunter, Hurler)
Chediak-Higashi	Large Lysosomes (Fusion of Primary Granules)	Albinism, ↑Susceptibility to Infection
May-Hegglin	Large Platelets, ↓Number, Döhle Bodies in Segs, Monos, and Lymphs	Does Not Affect Leukocyte Function
Pelger-Huet	Hyposegmented Polys	Normal Function

MYELOPROLIFERATIVE DISEASES

1. **Myelodysplastic Syndromes- neoplastic, clonal,stem cell disorders characterized by cytopenias and BM dyspoiesis**
   a. **Refractory Anemia (*RA*) - <5% blasts**
   b. **Refractory Anemia with sideroblasts *(RARS)* - <5% blasts with ringed sideroblasts**
   c. **Refractory Anemia with excess blasts (*RAEB*) - 5-20% blasts**

2. **Myeloproliferative Neoplasms - neoplastic, clonal disorders, characterized by increases in red cells, WBCs and/or platelets**
   a. **Primary Myelofibrosis (*PM*) - "dry tap", tear- drop shaped rbc, bone marrow fibrosis**
   b. **Essential Thrombocythemia (*ET*) - ↑megakaryocytes, platelets (> *1 million/mm3)***
   c. **Chronic Myelogenous Leukemia (*CML*) - ↑myelocytic precursors (*from blast to mature neutrophil*), ↓LAP, Philadelphia chromosome [*Ph' , t(9;22), bcr/abl fusion gene*]**
   d. **Polycythemia Vera *(PV)* -pancytosis, Janus kinase 2 mutation (*JAK2*)**

Leukemoid Reaction vs. CML

CHARACTERISTIC	LEUKEMOID	CML
LAP	↑	↓
Toxic Granulation	Yes	No
Döhle Bodies	Yes	No
Ph'; bcr / abl	No	Yes

3. **Acute Myeloid Leukemias (*AML*)**
   a. **WHO classification (> *20% blasts*)**
      - ❖ ***AML with recurrent chromosomal abnormalities***
        - ☞ **t(8;21)**
        - ☞ **Inv (16) or t(16;16)**
        - ☞ **t(15;17)**
        - ☞ **11q23**
      - ❖ ***AML with dysplasia***
        - ☞ **may follow MDS**
      - ❖ ***AML & MDS therapy related***
      - ❖ ***AML not otherwise classified - defaults to the FAB classification***
      - ❖ ***AML of ambiguous lineage***
   b. **French, American, British (FAB) classification (>30% blasts)**

AML	PREDOMINANT CELL SEEN
M0	Myeloblast without differentiation
MI	Myeloblast with minimal maturation
M2	Myeloblast with maturation
M3	Promyelocyte (APL)
M4	Myeloblast and monoblast (AMMoL)
M5	Monoblast (AMoL)
M6	Erythrocytic series (*Erythroleukemia*)
M7	Megakaryocyte *(Megakaryocytic Leukemia*)

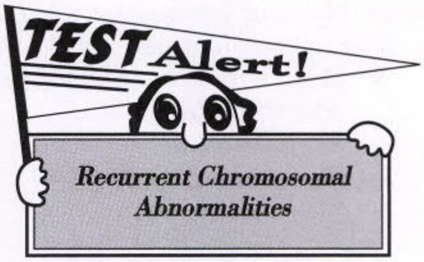
Recurrent Chromosomal Abnormalities

ABNORMALITY	ASSOCIATED WITH
t(15;17) Retinoic Acid Receptor α-gene	APL (Acute Promyelocytic Leukemia)
t(9:22) Philadelphia Chromosome	CML
t(8;21)	AML (M2)
t(16;16) or inv(16)	AML (M4Eo Variant)
11q23	AML (M5)

LYMPHOPROLIFERATIVE NEOPLASMS

1. **Acute lymphoblastic leukemia/ lymphoma *(ALL/LBL)***
   a. **Precursor B-cell**
      - ❖ *$CD\ 10^+$, $CD\ 19^+$, $CD\ 22^+$, $CD\ 24^+$*
   b. **Precursor T-cell**
      - ❖ *$CD\ 2^+$, $CD\ 4^+$, $CD\ 5^+$, $CD\ 8^+$*

2. **Chronic lymphoid neoplasms**
   a. **Chronic lymphocytic leukemia/ lymphoma (*CLL*)**
      - ❖ ***Mature lymphocytes (soccer ball nucleus)***
      - ❖ *$CD\ 19^+$, $CD\ 20^+$, $CD\ 23^+$ **and** $CD\ 5^+$*
   b. **Hairy cell leukemia (*HCL*) -**
      - ❖ ***mature lymphocytes with cytoplasmic projections, pancytopenia, splenomegaly***
      - ❖ ***TRAP positive***
      - ❖ *$CD\ 103^+$, $CD\ 11c^+$, $CD\ 25^+$*



3. **Plasma cell neoplasms**
   - a. **Plasma cell myeloma (*Multiple Myeloma*)**
     - ❖ ***Bone pain (multiple lytic lesions)***
     - ❖ ***Sheets of plasma cells in BM***
     - ❖ ***Rouleaux - red cells appear as a "stack of coins" on blood smear***
     - ❖ ***↑ serum protein (IgG or IgA monoclonal spike), ↑ Ca^{++}***
     - ❖ ***Urinary excretion of light chains (Bence Jones protein)***
   - b. **Plasma cell leukemia**
     - ❖ ***>20% plasma cells in peripheral circulation***
   - c. **Waldenstrom macroglobulinemia**
     - ❖ ***Normal bone scan***
     - ❖ ***↑ IgM***
     - ❖ ***↑ serum viscosity***
4. **Hodgkin Lymphoma**
   - a. **Reed Sternberg cell**
   - b. **Stepwise predictable spread**
   - c. **Bi-modal incidence**

Plasma Cell Myeloma vs. Waldenstrom

CHARACTERISTIC	MULTIPLE MYELOMA	WALDENSTROM
Bone Involvement	Yes	No
Serum Viscosity	±	↑↑
Immunoglobulin	IgG (Bence-Jones)	IgM (Heavy Chain)

Special Cytochemical Stains/ Inclusions/ Markers

STAIN/ INCLUSION/ MARKERS	INDICATES:	SIGNIFICANCE:
Prussian Blue	Iron	Sideroblastic Anemia, Iron overload
LAP	Leukocyte Alkaline Phosphatase	↑ Leukemoid Reaction, P. vera; ↓CML
Peroxidase/Sudan Black	Myeloperoxidase/Lipid	Myeloid Precursors Pos / Lymphoid Precursors Neg
Specific Esterase	Granulocyte Precursors	Negative in Monocytic Leukemia
Non-Specific Esterase	Monocyte Precursors	Positive in Monocytic Leukemias
TRAP	Tartrate-Resistant Acid Phosphatase	Hairy Cell Leukemia
Auer rods	Coalition of 1° Granules	Acute Myeloid Leukemia
CD13, CD33	Myeloid Lineage	Myeloid / Monocytoid Leukemias
CD41, CD42, CD61	Megakaryocytes	Megakaryocytic Leukemia
CD14, CD64	Monocyte lineage	Monocytoid Leukemias
CD2, CD3, CD5, CD7	T-lineage	T-cell Neoplasms
CD10 (CALLA), CD19, CD22	B-lineage	B-cell Neoplasms
CD34	Stem Cells	Stem Cells for Transplantation, Acute Leukemia
CD71	Transferrin receptor	Erythroleukemia
CD45	Common Leukocyte Antigen	Found on all Leukocytes
CD103 , CD11c, CD25	HCL	Also seen in other Lymphoproliferative Disorders
JAK 2 Mutation	PV	Also seen in other MPN

Lysosome and Lipid Storage Disorders

DISEASE	ACCUMULATED LIPID	LAB DIAGNOSIS
Gaucher	Glucocerebroside	BM Macrophages with wrinkled or striated cytoplasm
Niemann-Pick	Sphingomyelin	BM Macrophages with globular or foamy cytoplasm, Sea-blue Histiocytes

REMEMBER!

Tartrate-Resistant Acid Phosphatase for Hairy Cell Leukemia

T.R.A.P. the Hairy Beast

Differences in Multiple Myeloma and Waldenstrom

Stains/ Inclusions/ Markers Associated with Various Disorders

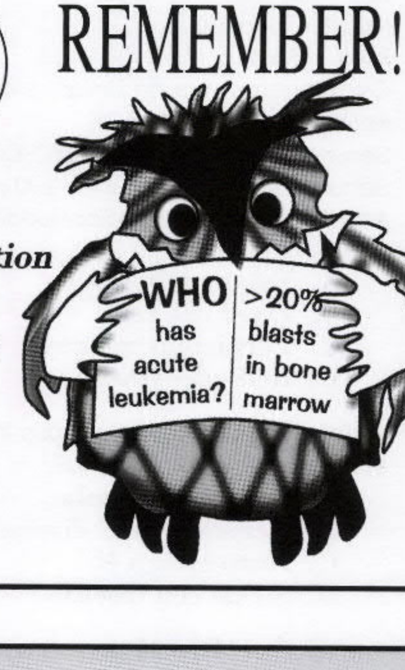
REMEMBER!

JAK 2 mutation and P. vera

JAcK loves Poly Vera

REMEMBER!

WHO Classification of Acute Leukemia



***Be able to identify mature, immature, and abnormal RBCs and WBCs from graphic images.
Correlate abnormal findings with appropriate disease states.***

HEMATOLOGY SAMPLE QUESTIONS

1. The major iron storage compound is
   - **A. Hemosiderin**
   - **B Ferritin**
   - **C. Siderotic granules**
   - **D. Transferrin**

2. How would the following results on a 32 year old adult female be interpreted?

   Hemoglobin - 9.0 gm/dL
   MCV - 74 fL
   MCH - 27 pg
   MCHC - 30.0 g/dL
   RDW - 19.0 %
   Serum ferritin - 4 ng/mL (N=20-250 ng/mL)
   Serum iron - 29 g/dL (N=70-200 g/dL)
   TIBC - 590 g/dL (N=250-435 g/dL)
   % Saturation - 5

   - **A. Anemia of chronic inflammation**
   - **B. Iron deficiency anemia**
   - **C. Thalassemia minor**
   - **D. Sideroblastic anemia**

3. How would the following results on a 72 year old adult female be interpreted?

   Hemoglobin - 6 g/dL
   MCV - 114 fL
   MCH - 39 pg
   MCHC - 34 g/dL
   RDW - 18.5 %
   Oval macrocytes on Wright stain
   Reticulocyte count - 1.2%
   Serum B12 - 55 pg/ml (N=200-1000 pg/mL)
   Serum folate - 7 ng/ml (N=2-10 ng/mL)
   Anti-IF *(intrinsic factor)* antibodies - positive

   - **A. Folate deficiency**
   - **B. Liver disease**
   - **C. Pernicious anemia**
   - **D. Reticulocytosis**

4. Which of the following results from decreased synthesis of globin chains?
   - **A. Beta-thalassemia**
   - **B. Hemoglobin C disease**
   - **C. Hemoglobin M**
   - **D. Sickle cell disease**

5. The normal M:E ratio for an adult is
   - **A. 1:1.5**
   - **B. 3:1**
   - **C. 5:1**
   - **D. 9:1**

6. A patient with a negative dithionite solubility test has a band in the A region and a band in the S region on cellulose acetate hemoglobin electrophoresis at pH 8.6. On citrate agar there is only a band in the A region. Which of the following is compatible with these results?
   - **A. Hb AS**
   - **B. Hb AE**
   - **C. Hb AD**
   - **D. Hb** $\text{AC}_{\text{Harlem}}$

7. The failure of granulocytes to develop past the "band" or two-lobed stage is characteristic of
   - **A. Bernard-Soulier syndrome**
   - **B. Chediak-Higashi syndrome**
   - **C. May-Hegglin anomaly**
   - **D. Pelger-Huet anomaly**

8. A patient with an elevated WBC count with neutrophilia, a left shift, toxic granulation, vacuoles, dohle bodies and an increased LAP probably has which of the following?
   - **A. Acute myelogenous leukemia**
   - **B. Chronic myelogenous leukemia**
   - **C. Bacterial sepsis**
   - **D. Viral sepsis**

9. The following results were obtained on an automated CBC.

   Hemoglobin -11.2 g/dL
   Hct - 27%
   RBC - 2.1 X 10^6/uL
   MCV - 128 fL
   MCH - 53.3 pg
   MCHC - 41.5 g/dL
   RDW - 19.0 %

   All results are flagged. The technologist found no evidence of clots in the sample. What should be done next?

   - **A. Ask for a redraw**
   - **B. Warm the sample to 37° C and rerun**
   - **C. report the results if controls are in range**
   - **D. Rerun the sample and sign out if results match**

10. Plasma cells evolve from which cell line?
    - **A. Lymphocytic**
    - **B. Monocytic**
    - **C. Myelocytic**
    - **D. Megakaryocytic**

11. In performing a manual white blood cell count, 0.02 mL of blood was diluted with 1.98 mL of ammonium oxalate. An average of 50 cells were counted using a Neubauer hemacytometer. What is the patient's white count?
    - A. 5,000/µL
    - B. 5,500/µL
    - C. 10,000/µL
    - D. 11,000/µL

12. A 4 mL EDTA tube was received in the laboratory containing approximately 1 mL of whole blood. If performed on this sample, which of the following manual laboratory tests is most likely to be affected?
    - A. Hemoglobin
    - B. Retic count
    - C. Sed rate
    - D. WBC count

13. A peripheral blood smear stained with Prussian blue demonstrates siderocytes. On a Wright stained smear, what would be expected?
    - A. Basophilic stippling
    - B. Howell Jolly bodies
    - C. Heinz bodies
    - D. Pappenheimer bodies

14. A bone marrow differential performed on a patient showed 20% blasts. Flow cytometry studies demonstrated the blasts to be positive for CD10, CD19, CD22, and negative for CD13 and CD33. Which of the following diseases is most compatible with these findings?
    - A. ALL
    - B. AML
    - C. CML
    - D. CLL

15. Which of the following is diagnostic of acute promyelocytic leukemia?
    - A. t(9;22)
    - B. t(15;17)
    - C. t(16;16)
    - D. t(8;21)

16. Plasma cell (multiple) myeloma may be suspected if which of the following is seen on a peripheral smear?
    - A. Basophilic stippling
    - B. Bizarre blast cells
    - C. Hypersegmented neutrophils
    - D. Rouleaux

17. Which parameter is most likely affected by lipemia?
    - A. MCV
    - B. WBC count
    - C. Hemoglobin
    - D. RBC count

ANSWERS AND RATIONALE

1. B

Option A is a long-term water-insoluble iron storage compound but not the major one. Hemosiderin can be found in found in macrophage lysosomal membranes and seen in bone marrow aspirates stained with Prussian blue. Option C are iron inclusions found in red cells stained with Prussian Blue. Option D is the transport protein specific for iron.

2. B

An RDW greater than 14.5%, decreased iron/increased TIBC and greatly decreased ferritin (*indicating no iron stores*) support this diagnosis. Option A is incorrect because ferritin would NOT be decreased and the TIBC would be decreased. Option C is incorrect because in thalassemia minor, the Fe and TIBC would probably be normal and the anemia would be less severe. Also, the RDW would proabably be in the normal range. Option D is incorrect because the ferritin would be increased as would the serum iron.

3. C

Oval macrocytes, decreased B12 and positive IF antibodies are all indicators of pernicious anemia. Option A is incorrect because the folate is normal. Option B is incorrect because the anti-IF antibodies would NOT be positive. Option D is incorrect because of the normal reticulocyte count and the additional abnormal data.

4. A

All other options result from structural abnormalities.

5. B

The normal M:E ratio is between 3:1 and 4:1.

6. C

Hb AS would give a positive solubility and would show a separate band in the S region on citrate agar. Hb E would give a negative solubility test but would migrate to the C position on cellulose acetate. Hb C_{Harlem} would give a positive solubility test and would migrate to the C position on cellulose acetate.

7. D

Option A is a platelet adhesion problem characterized by giant platelets. Option B is characterized by giant lysosomes in leukocytes. Option C is characterized by giant platelets and Döhle bodies.

8. C

Option A is incorrect because there would be a predominance of lymphocytes and an LAP would not be performed. Option B is incorrect because the LAP score would be decreased. Option D would show a normal WBC count and LAP score with no dohle bodies, toxic granulation or vacuoles seen.

9. B

These results violate the "Rule of Three" and are strongly suggestive of a cold agglutinin. Warming the sample to 37°C will usually cause the agglutination to disperse. Option A would not be the first course of action. Options C and D are not acceptable because the results are flagged and indicate some type of interference or erroneous result.

10. A

Plasma cells evolve from B cells which are lymphocytes.

11. B

The formula for calculating manual cell counts is as follows:

$$\# \text{ cells counted} \times \frac{1}{\text{tot vol}} \times \text{dilution factor}$$

The dilution is 1:100. So the dilution factor is 100. volume factor can be eliminated by taking 10% of the number of cells counted and adding this to the number of cells counted.

$$50 \times .1 = 5$$
$$50 + 5 = 55$$

So,

$$55 \times 100 = 5{,}500/\text{mm}^3$$

12. C

Underfilling results in excess EDTA causing the red cells to shrink. This would cause the sed rate to be falsely decreased since the smaller cells will settle out more slowly. The other values would probably not be affected.

13. D

Siderotic granules are composed of iron and on a Wright stained smear appear as Pappenheimer bodies within the red cell. They are frequently seen in sideroblastic anemia, alcoholism, thalassemia and some preleukemic states. Option A is composed of RNA remnants and does not stain with prussian blue. It is associated with lead poisoning, thalassemia, and hemolytic anemias. Option B is composed of DNA remnants and is associated with hyposplenism, pernicious anemia and thalassemia. Option C is denatured hemoglobin, is NOT seen on a Wright stained smear and is associated with G6PD deficiency, exposure to oxidizing drugs, alpha thalassemia, and unstable hemoglobins.

14. A

>20% blasts in the bone marrow is associated with acute leukemias. CD10, CD19 and CD22 are indicative of B- lineage ALL. Option B would be positive for CD13 and CD33. Options C and D would not have >20% blasts in bone marrow.

15. B

The t(15;17) translocation is diagnostic of APL

16. D

Rouleaux due to increased plasma proteins (*monoclonal immunoglobulin*) may be seen in plasma cell myeloma. The serum viscosity is increased and the albumin:globulin ratio is decreased. Option A is seen in conditions associated with disturbed erythropoeisis. Option B is seen in leukemia states. Option C is seen in pernicious anemia.

17. C

Options A,B and D are measured by the impedence principle and are not affected by lipemia. Hemoglobin is measured optically and lipemia will cause a false increased value.

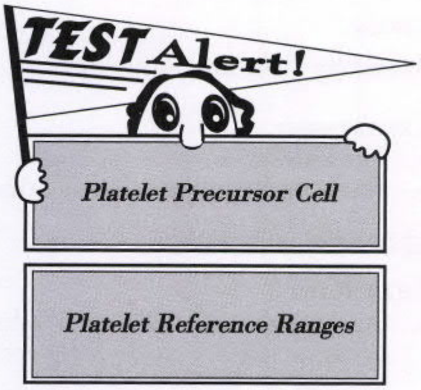
COAGULATION

by Daniel Haun

Platelets

PRODUCTION

1. Produced from megakaryocytes
2. Distribution
   a. 30% spleen
   b. 70% peripheral blood
   c. Reference range - 150,000-400,000/mm3
   d. Life span - 9-12 days



FUNCTIONS

1. Initial arrest of bleeding and formation of the platelet plug
   a. Adhesion
      - ❖ *Glycoprotein Ib binds to exposed collagen*
      - ❖ *Requires von Willebrands factor*
      - ❖ *Results in release (secretion) of ADP and other granule components (including Factor V and fibrinogen)*

   b. Aggregation
      - ❖ *Other platelets are stimulated by ADP to undergo shape change (disc to sphere to pseudopods) exposing the glycoprotein IIb / IIIa complex which binds fibrinogen (this is the complex that is blocked by a number of GP IIb/IIIa inhibitor drugs)*
      - ❖ *Fibrinogen binding links the platelets; the first (and reversible) phase of aggregation*
      - ❖ *With weak stimuli, the aggregates can disassociate but strong stimuli cause the aggregating platelets to release (secrete); with release, the aggregation is irreversible*

The bottom line..?

After adhesion and aggregation a platelet plug is built at the injury site. The PFA test asks the important question:
Do the platelets properly adhere and aggregate at the injury site?

2. Localization of the platelet plug
   a. Secreting platelets release arachidonic acid which converts to prostaglandin, (*becomes Thromboxane A2*) in the platelet
   b. Arachidonic acid is processed by adjacent endothelial cells to form platelet-inhibiting prostacyclin

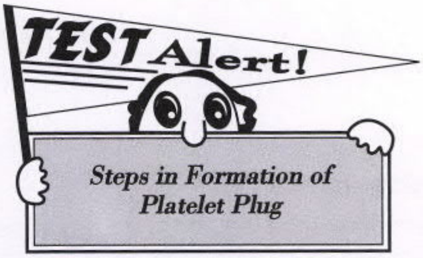
The bottom line..?

The platelet plug is limited to the injury site.

3. Assembly and localization of the fibrin clot
   a. Platelet release components include fibrinogen, Factor V and Factor VIII
   b. Fibrinogen is bound on the platelet surface during aggregation
   c. Factor VIII is bound to the platelet surface with von Willebrand factor
   d. Shape change exposes platelet membrane phospholipid (*PL*); the template for the assembly of the factor complexes
      - ❖ *Historically called Platelet Factor 3*
      - ❖ *Binds the Factor VIII and IXa complex (requires Ca^{++}) - no wonder Hemophilia A and Hemophilia B (Factor VIII and IX deficiencies) are clinically identical*
      - ❖ *Binds the Factor V and Xa complex; also requires Ca^{++}*

The bottom line..?

The platelet plug is a wonderful place to produce a fibrin clot and without the platelet presence, the fibrin won't form. It is clot promotion and clot localization all in one.


TEST Alert!
Steps in Formation of Platelet Plug

Plasma Coagulation Factors

FUNCTIONS

1. Substrate - Factor I (fibrinogen)

2. Cofactors - accelerate enzymatic reactions
   a. Factors III, V and VIII
   b. Factor HMWK (*high molecular weight kininogen*)

3. Enzymes
   a. Serine proteases - cleave peptide bonds (*Factors II, VII, IX, X, XI and XII*)
   b. Transamidase - XIII only

CHARACTERISTICS OF COAGULATION PROTEINS

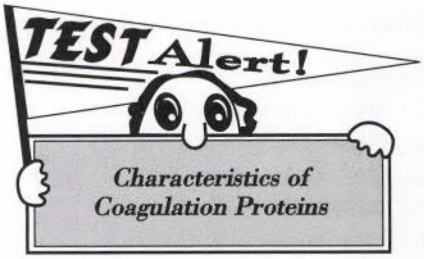
Coagulation Groups

GROUP	CONTACT	PROTHROMBIN	FIBRINOGEN
Factors	XI, XII, PK, HMWK	II, VII, IX, X	I, V, VIII, XIII
Vitamin K Dependent	No	Yes	No
Consumed in Clotting	No	No (except for II)	Yes

1. Contact proteins
   a. Factors XII, XI, PK (*prekallikrein*) and HMWK (*high molecular weight kininogen*)
   b. Participate in the initial phase of the intrinsic system
   c. NOT consumed during clotting (*found in both serum and plasma*)
   d. NOT Vitamin K dependent

2. Prothrombin proteins
   a. Factors II, VII, IX, X
   b. Vitamin K dependent
   c. NOT consumed during clotting *(except II)*
   d. Present in fresh and stored plasma and serum

3. Fibrinogen proteins
   a. Factors I, V, VIII, XIII
   b. Consumed during clotting (therefore not in serum)
   c. ↑ in acute phase (*pregnancy and inflammation*)



FACTOR NAMES

1. Noted by Roman numerals

2. Exceptions:
   a. Prekallikrein
   b. High molecular weight kininogen

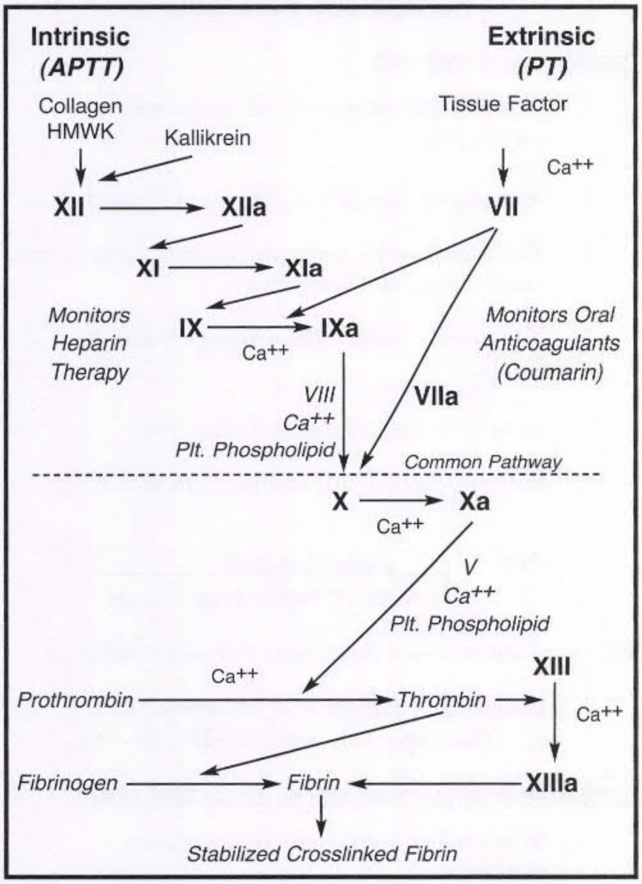
STABILIZED CROSSLINKED FIBRIN

1. Turn it on
   a. Activated intrinsically by the collagen (*via Factor XII*) contact system
   b. Activated extrinsically by disrupted endothelial cell membrane (*tissue factor or tissue thromboplastin*) complex with Factor VII to directly activate Factor X

2. Cofactors
   a. Factor VIII is bound with activated Factor IX by calcium to the platelet phospholipid membrane (*PL*)
   b. Together these factors activate Factor X *(in the common pathway Factor V is the cofactor to Factor Xa in a similar arrangement with calcium and PL*)
   c. This prothrombinase complex converts prothrombin to the active thrombin

3. Thrombin
   a. Cleaves peptides off of the fibrinogen molecule to form fibrin which polymerizes to form insoluble fibrin strands
   b. Thrombin also activates Factor XIII which crosslinks the fibrin strands at the "D" region (*birth of the D-dimer)*

Coagulation Cascade


Intrinsic
(APTT)
Extrinsic
(PT)
Collagen
HMWK
Kallikrein
Tissue Factor
Ca++
XII
XIIa
VII
XI
XIa
Monitors Heparin Therapy
IX
IXa
Ca++
Monitors Oral Anticoagulants (Coumarin)
VIII
Ca++
Plt. Phospholipid
VIIa
Common Pathway
X
Xa
Ca++
V
Ca++
Plt. Phospholipid
XIII
Ca++
Prothrombin
Thrombin
Ca++
Fibrinogen
Fibrin
XIIIa
Stabilized Crosslinked Fibrin

Other functions of thrombin

- Feeds back to "potentiate" factors V and VIII
- Recruits and aggregates platelets
- Turns on endothelial cell thrombomodulin *(receptor/activator for Protein C and Protein S system)* to inactivate Factors V and VIII

4. Turn it off
   a. Heparan sulfate on the endothelial cell binds antithrombin (*AT*) which inactivates the activated serine proteases (***heparin works this way, too!***)
   b. Activated Protein C and its cofactor Protein S (***when bound to its receptor/activator thrombomodulin***) inactivates Factors VIII and V

TEST Alert!
Knowledge of Coagulation Cascade

FIBRINOLYTIC SYSTEM

1. Turn it on
   a. Activated intrinsically by collagen via the Factor XII / contact pathway that initiates intrinsic clotting or extrinsically by tissue plasminogen activator (*TPA*)
   b. Activators convert the precursor plasminogen to plasmin

2. What does it do?
   a. Plasmin cleaves fibrin strands to soluble fragments of fibrin (***fibrin degradation products are X, Y, D or E***)
   b. Can come from fibrin clot (*fibrinolysis*) or from unclotted fibrinogen (*fibrinogenolysis)*
   c. D-dimer comes from crosslinked clot *(clot specific*)

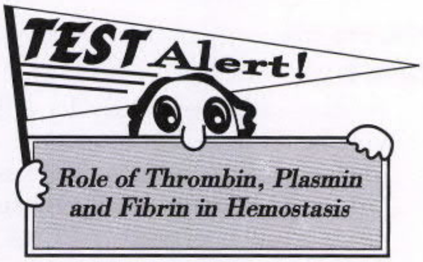
3. Turn it off
   a. TPA inactivated by tissue plasminogen activator inhibitor (*TPAI*) – stops activation
   b. Active plasmin inhibited by Alpha-2 Plasmin Inhibitor if it escapes the area of the clot. This prevents fibrinogenolysis

Excessive and inappropriate fibrinolysis

- Major feature of disseminated intravascular coagulation *(DIC)*; response to excessive clotting
- Also seen in liver disease *(activators are not cleared and the inhibitors are diminished)*
- Complications of cancer or surgery of the prostate or urinary tract where urokinase can leak into the circulation

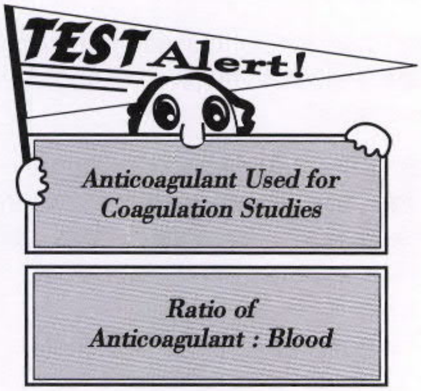
"Clotbusters"

- Streptokinase *(commercial)*
- Urokinase *(commercial)*
- Tissue plasminogen activator *(commercial and in vivo)*


TEST Alert!
Role of Thrombin, Plasmin and Fibrin in Hemostasis

Specimen Collection/Handling

1. Sodium citrate 3.2% (*Ca^{++} is necessary for both coagulation and platelet aggregation studies*)
2. Whole blood - anticoagulant ratio = 9:1
3. Use plastic tubes or siliconized glassware (*glass activates factor XII and platelets will adhere to glass*).
4. Coagulation samples can now be drawn first when using evacuated tubes (*vacutainers*). Historical reports of contamination with tissue thromboplastin during collection are unfounded.
5. Hemolyzed samples should NOT be used for platelet aggregation studies (*red cells contain ADP*).
6. Lipemic samples may cause problems with coagulation and aggregation studies (*may obscure changes in optical density*).


TEST Alert!
Anticoagulant Used for Coagulation Studies
Ratio of Anticoagulant : Blood

Routine Tests of Hemostatic Function

PROTHROMBIN TIME (*PT*)

1. Screen for extrinsic & common pathways
2. Measures factors I, II, V, VII and X
3. Monitors oral anticoagulants (warfarin, coumarin, dicoumarol)
4. Reagent - tissue thromboplastin & $CaCl_2$
5. Sensitive to Vitamin K factors
6. International normalized ratio: INR

$$\mathrm{INR} = \left(\frac{\text{patient result}}{\text{mean of reference range}}\right)^{\mathrm{ISI}}$$

(ISI = International Sensitivity Index from manufacturer)

7. Reference range = < 13 seconds
   a. Therapeutic goal: INR 2.0 - 3.5

ACTIVATED PARTIAL THROMBOPLASTIN TIME (APTT)

1. Screen for intrinsic & common pathways
2. Measures all factors except VII and XIII
3. Monitors heparin therapy
4. Reagents - activator (kaolin, celite or ellagic acid), platelet phospholipid (*PL*) & $CaCl_2$

TEST Alert!
Therapy Monitored by PT and PTT

REMEMBER!

Monitoring by PT and APTT

APTT=2 T's Together Remind You of an "H"=Heparin

PT=Coumarin; Vitamin K Factors

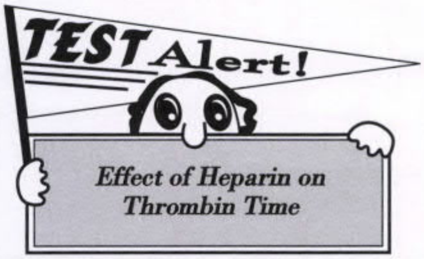
5. Reference range = 20 - 40 seconds
   a. Therapeutic goal: 1.5-2.5 times "normal" or use Heparin Response Curve

FIBRINOGEN ASSAY

1. Quantitative measure of Factor I
2. Reference range = 200-400 mg/dl

THROMBIN TIME (*TT*)

1. Does NOT measure defects in intrinsic/extrinsic pathways


TEST Alert!
Effect of Heparin on Thrombin Time

2. Affected by ↓ fibrinogen levels and presence of heparin and other antithrombins
3. Reference range = < 20 seconds

BLEEDING TIME

1. Historically measured platelet function and numbers but generally replaced by PFA

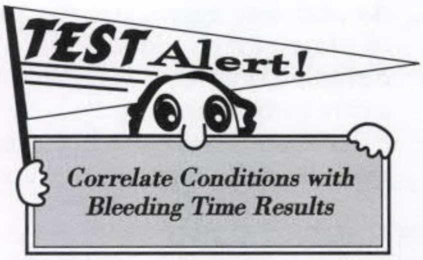
PLATELET FUNCTION ASSAY (PFA TEST)

1. Measures platelet function with collagen, ADP and epinepherine
2. Sensitive to aspirin, vWD, and ADP receptor problems
3. Replaces the bleeding time as a screen

CLOT RETRACTION

1. Evaluates platelet function, fibrinogen, red cell volume and fibrinolytic activity
2. Abnormal if platelet count <100,000/mm^3

TEST Alert!
Implication of a Rapidly Dissolved Clot


TEST Alert!
Correlate Conditions with Bleeding Time Results

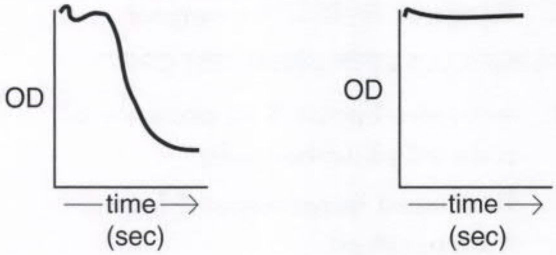
3. Anemia and hypofibrinogenemia ↓ clot retraction
4. Rapid dissolution of clot indicates ↑ fibrinolytic activity (example = DIC)
5. Glanzmann Thrombasthenia - no clot retraction

Special Tests of Hemostatic Function

PLATELET AGGREGATION

1. Necessary for platelets to stick to each other
2. In vivo aggregating agents
   a. ADP
   b. Collagen
   c. Epinephrine
   d. Thrombin
   e. Serotonin
   f. Arachidonic acid
   g. Ristocetin
   h. Snake venom
   i. Antigen-antibody complexes
   j. Fibrin degradation products (FDPs)
3. In vitro aggregating agents
   a. ADP
   b. Collagen
   c. Ristocetin
   d. Epinephrine
   e. Thrombin
   f. Arachidonic acid
4. Measured with photo-optics

Platelet Aggregration



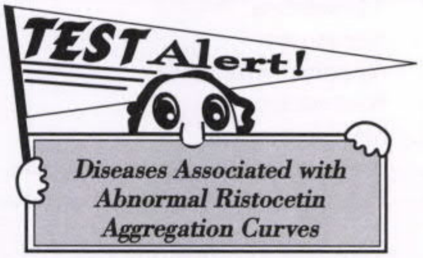
  - a. As platelets aggregate, the turbidity of platelet rich plasma ↓
  - b. Samples kept at RT, heated to 37C prior to testing
  - c. Stirring necessary to bring platelets in contact for aggregation to occur

5. Aspirin inhibits secondary wave of aggregation (*destroys cyclooxygenase*)

6. Abnormal ristocetin-induced aggregation
   - a. Bernard-Soulier
   - b. von Willebrand

Differentiating Platelet Disorders

DISORDER	NUMBER	MORPHOLOGY	ASSAYS
Glanzmann	N	N	Abn Agg (all agents ↓) vWF N
Bernard-Soulier	Moderate ↓	Giant Platelets	Abn Adhesion (ristocetin ↓) vWF N
von Willebrand's	Usually N	N	Abn Adhesion (ristocetin ↓ vWF Abn



Diseases Associated with Abnormal Ristocetin Aggregation Curves

Effect of Aspirin on Platelet Aggregation Curve

FACTOR ASSAYS

1. PT and APTT tests performed with specific factor deficient plasma

2. % activity and amount of correction with normal plasma determined

3. Range of 40-150 % = normal

DILUTE RUSSELL'S VIPER VENOM TEST (DRVVT)

1. Activates Factor X in presence of reduced phospholipids

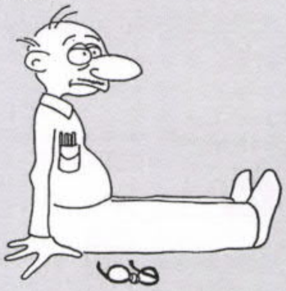
2. Prolonged in presence of Lupus Anticoagulant

REMEMBER!

Platelet Adhesion Disorders

Wimpy Willie *(von Willebrand's Disease)*

- normal platelets
- ↓Factor VIII
- ↓ vWF



Super Bernie *(Bernard-Soulier)* = **GIANT** Platelets

ANTI-FACTOR Xa ASSAY

1. Monitors Low Molecular Weight Heparin therapy (*LMWH)* and other antia Xa oral anticogulants

2. *The APTT is insensitive to LMWH*

REPTILASE TIME

1. Snake venom enzyme

2. Test similar to thrombin time, but is not inhibited by heparin (*good to use for patients on heparin*)

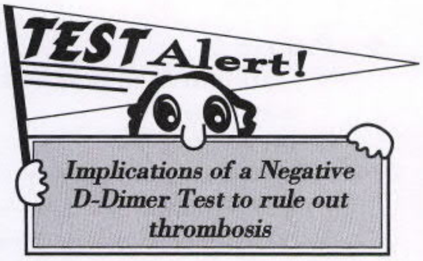
3. Reference range = 18-22 seconds

FIBRINOLYSIS TESTING

1. Fibrin/fibrinogen degradation *(split)* products *(FDP/FSP)*
   - a. Latex beads coated with anti-fibrinogen
   - b. Historical use as positive screen in DIC

2. Latex D-Dimer - monoclonal antibody to crosslinked D fragment; positive in DIC, deep vein thrombosis and pulmonary embolism

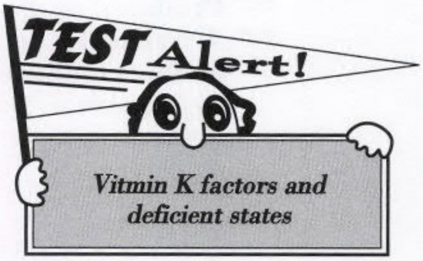
3. Quantitative D-dimer- has negative predictive value to rule out thrombotic events



Implications of a Negative D-Dimer Test to rule out thrombosis

Hemostasis Disorders

1. Inherited Disorders
   - a. Hemophilia A
     - ❖ *Deficiency of Factor VIII*
     - ❖ *Sex linked recessive - almost exclusive to men*
     - ❖ *Spontaneous bleeding into joints*
     - ❖ *Treat with commercial Factor VIII*
   - b. Hemophilia B
     - ❖ *Deficiency of Factor IX*
     - ❖ *Sex linked recessive - almost exclusive to men*
     - ❖ *Clinically identical in inheritance and symptoms to Hemophilia A*
     - ❖ *Treat with Factor IX concentrates*
   - c. Hemophilia C
     - ❖ *Deficiency of Factor XI*
     - ❖ *Incomplete autosomal recessive*
     - ❖ *Wide range in clinical severity*
     - ❖ *High incidence in Ashkenazi Jews*
     - ❖ *Only contact factor associated with bleeding*
   - d. von Willebrand Disease
     - ❖ *Primary defect in the von Willebrand Factor (vWF)*
     - ❖ *vWF binds platelets via the Glycoprotein 1B/V/IX receptor*
     - ❖ *Usually find secondary deficiency of Factor VIII*
     - ❖ *Autosomal dominant - males and females are affected equally*
     - ❖ *Platelet adhesion defect with abnormal PFA test*
     - ❖ *Treat with cryoprecipitate or DDAVP (drug); Factor VIII concentrates do NOT contain vWF*
   - e. Factor XIII deficiency
     - ❖ *Autosomal recessive*
     - ❖ *NOT detected by common coagulation tests*
     - ❖ *Results in a significant bleeding disorder ; poor wound healing*
     - ❖ *Detected with the 5M urea test*
2. Acquired Disorders
   - a. Inhibitors - usually IgG antibody directed against a specific factor or phospholipids (*PL*)
     - ❖ *Lupus anticoagulant (LA)*
       - ☞ Directed against phospholipids
       - ☞ Seen in Lupus Erythematosis (*about 5-10%*) but also seen in malignancies, infections, drug therapy and other autoimmune disorders
       - ☞ Screening tests use low concentration phospholipid reagents (*e.g. DRVVT*)
       - ☞ Use platelet neutralization techniques to confirm presence (*PL neutralizes antibody and test will correct*)
       - ☞ Can prolong the APTT
       - ☞ Predisposes patient to clotting
     - ❖ *Factor VIII inhibitor - most common specific inhibitor, but others have been demonstrated; APTT mixing studies differentiate factor deficiency from inhibitor*
       - ☞ If corrected by normal plasma, a factor deficiency is indicated
       - ☞ If NOT corrected, an inhibitor should be investigated
   - b. Vitamin K deficiency
     - ❖ *Functional deficiency of Factors II, VII, IX and X*
     - ❖ *PIVKA molecules (proteins in vitamin K absence) - present but not functional*
     - ❖ *Vitamin K originates from diet and bacteria in gut*
     - ❖ *Deficiency seen in poor diet and with broad spectrum antibiotic use*
     - ❖ *Same result observed in warfarin therapy*



Vitmin K factors and deficient states

Fibrinolysis

	PRIMARY	SECONDARY (DIC)
Platelet Count	N	↓
Red Cell Morphology	N	RBC Fragments
PT and APTT	Abnormal	Abnormal
FDP	+	+
D-Dimer	—	+

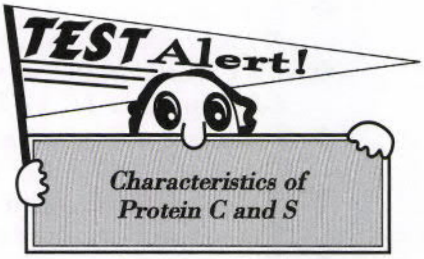
c. Liver Disease (*depending on severity or stage*)
  - *Deficiency of Factors I, II, V, VII, IX and X*
  - *Factor VII deficiency most pronounced*
  - ↓ *clearance of plasminogen activators*
  - ↑ *FDP due to fibrin(ogen)olysis*

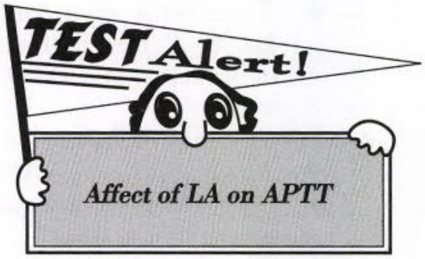
d. Disseminated Intravascular Coagulation (*DIC*)
  - *Secondary to sepsis, obstetric complications, Ebola virus infection*
  - *Thrombotic occlusion of micro-circulation*
  - *RBC fragments*
  - *Consumption of platelets and Factors I, V, VIII*
  - *High levels of FDP and D-dimer*

Thrombotic Diseases

1. Arterial Events - platelet driven *(arthlerosclerosis, prosthetic heart devices)*

2. Venous Events
   a. Blood flow problems *(superficial or deep vein thrombosis)*
   b. Clot inhibitor deficiency *(about 20%)*
      - *Antithrombin (formerly ATIII)*
        - Principle antagonist of active coagulation proteases
        - Produced in the liver
        - Activated by heparan sulfate on the endothelial cell and by heparin as therapeutic drug
      - *Protein C*
        - Vitamin K dependent serine protease
        - Activated by thrombomodulin on the endothelial cell
        - Requires Protein S cofactor
        - Inactivates Factors V and VIII
      - *Protein S*
        - Vitamin K dependent
        - Co-factor for Protein C
        - Bound and free state
        - Only free protein S is functional so measure total and free
   c. Factor V Leiden
      - *Mutant Factor V*
      - *Resists the action of Protein C/S*
      - *Activated Protein C Resistance Test*
   d. Antiphospholipid syndrome
      - *Any of three classes of antibodies*
        - Anticardiolipin antibodies
        - Lupus anticoagulant
        - Specific antibodies (*e.g. beta-2-glycoprotein)*
   e. Prothrombin mutation
      - *Mutation at position 20210*
      - *First described in 1996*
      - *1-2% of general population are heterozygotes*
      - *Results in* ↑ *thrombin formation*
      - ↑ *risk of thrombotic event*


TEST Alert!
Characteristics of Protein C and S


TEST Alert!
Affect of LA on APTT

Anticoagulant Therapy

1. Platelet inhibitors
   a. Aspirin- destroys cyclo-oxygenase to inhibit release reaction
      ❖ *Affected platelets are inhibited permanently until replaced*
   b. Clopidogrel (Plavix^{TM})- Blocks the ADP recptor (P2Y12) and is also permanent until platelet is replaced
   c. Glycoprotein IIb/IIIa receptor inhibitors (Abciximab, Eptifibatide,Tirofiban)- not permanent
2. Oral plasma protein anticoagulants
   a. Wafarin- prevents production of functional Factors II, VII, IX and X by inhibiting Vitamin K.
      ❖ requires monitoring using PT/INR
   b. Thrombin inhibitor (*Dabigatran*)- requires no monitoring
   c. Factor Xa inhibitors (*Rivaroxaban and Apixaban*)- generally require no monitoring
3. Parenteral anticoagulants
   a. Heparin (unfractionated)
      ❖ *Inhibits the active serine protease factors via Anti-thrombin*
      ❖ *Usually monitored with the APTT using a heparin response curve*
      ❖ *Can also be monitored using anti-Xa assay*
   b. Low Molecular Weight Heparin (LMWH)
      ❖ *Targets Factor Xa*
      ❖ *Yields a more predictable response than unfractionated heparin*
      ❖ *Does not usually require monitoring but if needed, monitor with the anti factor Xa assay.*

COAGULATION SAMPLE QUESTIONS

1. If one performs an APTT on a patient on high-dose warfarin therapy, we would expect that the result would be:
   A. Normal because warfarin effects the PT only
   B. ↑ because of fibrinogen split products
   C. ↑ because of factor VII deficiency
   D. ↑ because of other multiple factor deficiencies

2. Abnormal PFA results and giant platelets best describe
   A. Bernard-Soulier syndrome
   B. Glanzmann thrombasthenia
   C. von Willebrand disease
   D. Wiskott-Aldrich syndrome

3. A 7 year old male child is a candidate for ear tube surgery because of repeated ear infections but he has a persistent prolonged Prothrombin Time. The APTT is normal. Which condition is most likely?
   A. Congenital factor VII deficiency
   B. Vitamin K deficiency
   C. Hemophilia A
   D. Factor V Leiden

4. A 22 year old female was seen in the emergency room with evidence of bleeding following a spider bite. Laboratory results show

   Blood smear: Schistocytes
   Platelet count: 50,000/mm3
   PT: 20 secs
   APTT: 60 secs
   d-dimer: positive

   The most likely diagnosis is
   A. Allergic reaction
   B. Primary fibrinolysis
   C. Secondary fibrinolysis
   D. Vitamin K deficiency

5. A 4 year old male has a prolonged APTT of 53 seconds. Mixing with normal plasma at a ratio of 1 to 1 yields an APTT of 50 seconds. Which of the following is most likely?
   A. Systemic lupus erythematosus
   B. Inhibitor or other anticoagulant
   C. Hemophilia B
   D. Hemophilia A

ANSWERS AND RATIONALE

1. D

The prothrombin time is used to monitor warfarin therapy, but warfarin results in deficiencies of Factors II, VII, IX, and X. The correct answer is D because Factors II, IX and X are also measured by the APTT.

2. A

Options B and C are incorrect because platelet morphology is normal even though the bleeding time is prolonged. Option D is incorrect because this syndrome is characterized by tiny platelets and a prolonged bleeding time.

3. B

B is most likely because of the presentation. The child is likely treated with repeated courses of antibiotics which kills Vitamin K producing organisms. Congenital deficiencies are extremely rare since inheritance is autosomal recessive. Hemophilia A yields a normal PT and prolonged APTT and Factor V Leiden causes neither.

4. C

Option A is incorrect as allergies do not result in coagulation abnormalities. Option B is incorrect because schistocytes, decreased platelets and a positive D-dimer test are not seen in primary fibrinolysis. Option D is NOT characterized by schistocytes and a positive D-dimer test.

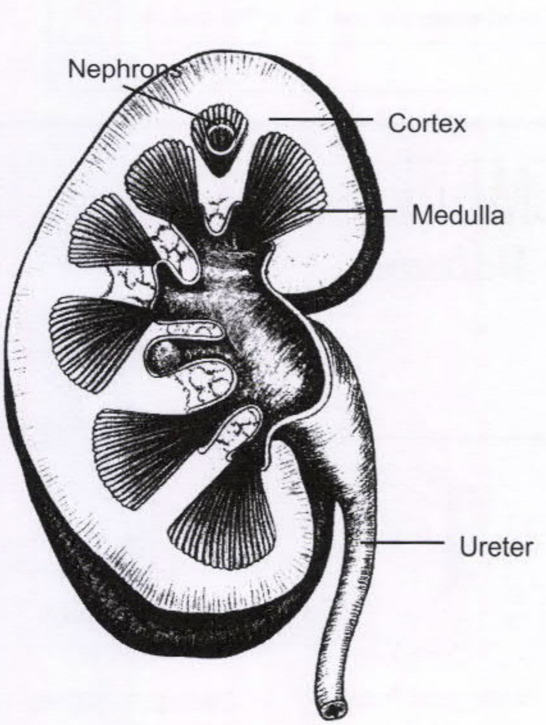
5. B

The 1 to 1 mix is still abnormal so the mixing study indicates a strong anticogulant or inhibitor. Option A is incorrect since the patient might have a “lupus anticoagulant” but that does not mean he has systemic lupus erythematosus (*SLE*). A small number of patients with SLE exhibit the lupus anticoagulant but it is also seen in many other conditions. Options C and D are both incorrect since a 1:1 mix would correct in either condition.

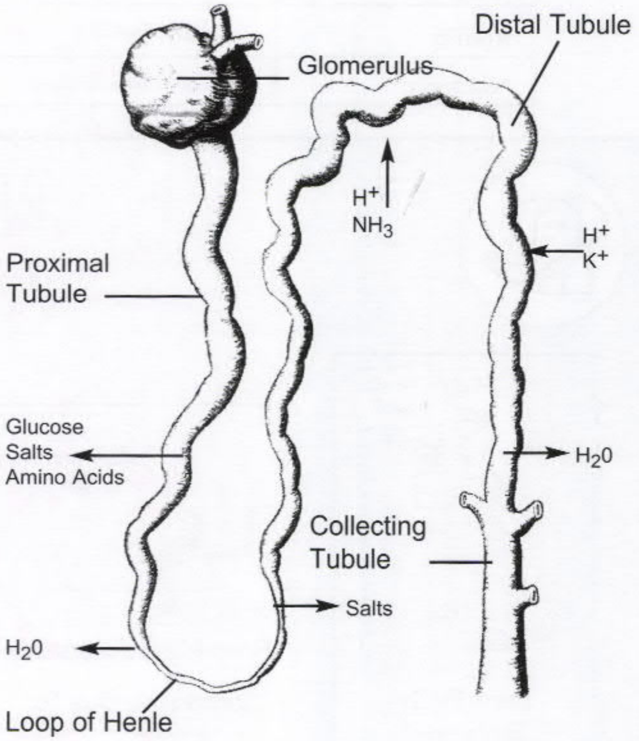
URINALYSIS/BODY FLUIDS

by Michele Zitzmann*

Kidney


Nephrons
Cortex
Medulla
Ureter

Nephron


Distal Tubule
Glomerulus
H^+
NH_3
H^+
K^+
Proximal Tubule
Glucose
Salts
Amino Acids
H_20
Collecting Tubule
Salts
H_20
Loop of Henle

• *Diagrams adopted with permission from Modern Urine Chemistry Manual;Siemens Healthcare Diagnostics Inc.; 1996*

Renal Physiology

NEPHRON

1. Major functional unit of the kidney
2. Approximately 1 million per kidney
3. Composed of glomerulus and renal tubules

GLOMERULUS

1. Coil of capillary vessels
2. Non-selective filter of plasma substances of less than 70,000 MW
3. Water, glucose, electrolytes, amino acids, urea, uric acid, creatinine, and ammonia comprise the glomerular filtrate
4. Filters 120 ml/min, or one-fifth of renal plasma

TUBULES	FUNCTION
Proximal Tubule	Reabsorbs water, Sodium Chloride, Bicarbonate, Potassium, Calcium, Amino Acids, Phosphates, Protein, and Glucose Glucose - Threshold Substance -Reabsorb at 160-180 mg/dl or less Secretes - Sulfates, glucuronides, Hydrogen ions and drugs
Loop of Henle Descending	Reabsorbs water No solutes reabsorbed
Loop of Henle Ascending	Reabsorbs Solutes (Sodium, Chloride, Calcium, and Magnesium No water reabsorbed
Distal & Collecting Tubules	Reabsorbs Sodium Secretes Potassium, Ammonia and Hydrogen ions Potassium ions exchanged for sodium ions

*original contribution by Libby Spence

URINE VOLUME

	VOLUME	DISEASE AND CAUSES
Normal	Average of 1200-1500 ml/day	
Polyuria	>2500 ml/day	Diabetes insipidus, Diabetes mellitus, diuretics Caffeine, alcohol, excessive water intake
Oliguria	<400 ml/day	Dehydration, Vomiting, Diarrhea, Burns, Perspiration
Anuria	Complete Cessation	Kidney Damage, Decrease blood flow to kidneys
Nocturia	Increase volume at night	

REMEMBER!

Urine Volume

Gotta Go Again!!
LADIES

Poor Polly (polyuria)

- Diabetes mellitus
- Diabetes insipidus

Which end to cover?

Poor Ollie (oliguria)

- Dehydration Due To:
  - *Vomiting*
  - *Diarrhea*
  - *Burns*

Nocturnal Ned (nocturia)

Absent Andy (anuria)

- Damage to Kidney
- ↓Blood Flow to Kidney *(↓Cardiac Output)*

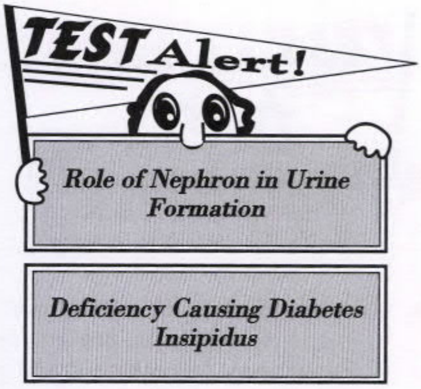
URINE COMPOSTITION

1. Urea *(non-protein nitrogen)*
   a. Metabolic waste product produced in liver from breakdown of protein
   b. 1/2 total urinary dissolved organic solids
2. Other organic solids *(non-protein nitrogens)*
   a. Uric acid
   b. Creatinine
3. Inorganic solids
   a. Chloride - primary constituent
   b. Na^+
   c. K^+
4. Water

HORMONES

HORMONES	SOURCE	ACTION
Aldosterone	Adrenal Cortex	Increases rate of sodium reabsorption
Arginine Vasopressin (AVP)*	Posterior Pituitary Gland	Reabsorption of water from the distal tubules Deficiency - Diabetes insipidus
Erythropoietin	Kidney	Stimulates production of erythrocytes

*** previously called Antidiuretic Hormone (ADH)**



Role of Nephron in Urine Formation

Deficiency Causing Diabetes Insipidus

Specimen Collection and Handling

COLLECTION METHODS

1. **Random**
   a. **Most common specimen type**
   b. **Easiest to collect**
   c. **Useful for routine screening tests**

2. **First morning**
   a. **First voided specimen upon waking**
   b. **Ideal screening specimen *(most concentrated)***

3. **Midstream clean catch**
   a. **Clean external genital area**
   b. **First and last stream of urine voided; midstream collected**
   c. **Specimen of choice for bacterial culture in routine circumstances**

4. **Catheterization**
   a. **Insertion of catheter directly into bladder via urethra**
   b. **Avoids external contamination - may introduce infection**

5. **Pediatric**
   a. **Sterile, plastic collection bag placed over genital area with adhesive**
   b. **Bag checked every 15 minutes**
   c. **Many sources of contamination**

6. **Suprapubic aspiration**
   a. **Insert needle through suprapubic abdominal area directly into bladder**
   b. **Avoids external contamination - may introduce infection**
   c. **Optimum specimen for bacterial culture; invasive procedure**

24 HOUR URINE

1. **Collected over 24-hour period**

2. **First specimen discarded while all others collected**

3. **Used for quantitative urine studies**

4. **Completeness of collection monitored by creatinine levels *(should be > 1.0 mg/dl)***

ANALYSIS

1. **Analyze within 1 hour of voiding *(NOT 1 hour after received in lab!)***

2. **Effects of prolonged sitting of specimens at room temperature**

⬆	⬇
Nitrite (bacterial growth)	Glucose (glycolysis due to bacteria and yeast)
pH (urea converted to ammonia)	Ketones (volatization - exposure to air)
Turbidity (bacterial growth, red or white cells, or amorphous material)	Bilirubin (exposure to light)
	Urobilinogen (oxidized to urobilin)
	Cells and Casts (lysis)
Changes in color occur due to oxidation or reduction of metabolites.	

Urine Changes that Occur Upon Standing & Best Preservative to Use

REFRIGERATION

1. **Preservation method of choice *(up to 24 hours)***

2. **May result in precipitation of amorphous crystals**

3. **After removal from the refrigerator, let sample return to room temperature before testing *(approximately 15 min)***

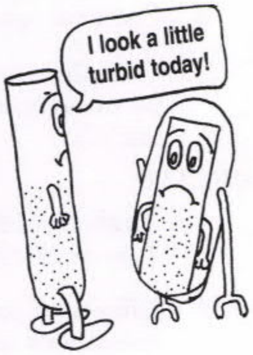
Physical Examination

ODOR

1. **Not evaluated, but may provide clue to constituents**

2. **Associations**
   a. **Fruity - ketones**
   b. **Ammonia - old urine**
   c. **"Mousy" - phenylketonuria *(PKU)***
   d. **Maple syrup - Maple Syrup disease *(branched chain aminoaciduria)***

CLARITY


I look a little turbid today!

1. Normal urine is clear
2. Any of the urinary elements *(cells, casts, crystals)*, or bacteria may make urine cloudy

COLOR

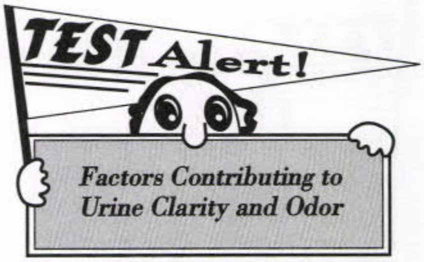
1. Normal urine is a pale yellow (*straw*) to yellow color
2. Urochrome gives urine its normal color

PH

1. Normal urine is slightly acid *(pH-6.0)*, Random *(4.5-8.0)*
   a. Postprandial sample *(2 hours after eating)*, urine may be alkaline *(alkaline tide)*

Color as a Clue

ABNORMAL COLOR	SUBSTANCE
Red	Hemoglobin Red Blood Cells Myoglobin Porphyrin Uroerythrin
Red-Brown	Hemoglobin Red Blood Cells Myoglobin
Yellow-Brown/Amber-Yellow-Green	Bilirubin Biliverdin
Yellow-Orange	Bilirubin Urobilin Pyridium (drug)
Bright Yellow	Vitamin C
Dark Yellow	Concentrated Specimen Bilirubin Urobilin
Brown-Black	Methemoglobin (oxydized RBC's) Homogentisic Acid (Alkaptonuria) Melanin
Blue	Indican (Tryptophane Metabolic Disorder)
Green/Blue-Green	Old Urine Psuedomonas
Port Wine	Porphyrin


TEST Alert!
Factors Contributing to Urine Clarity and Odor

b. When urine stands at room temperature *(or warmer)* for some time, it may become alkaline
c. Acidic - Metabolic or respiratory acidosis, high protein diet, cranberry juice
d. Alkaline-vegetarian

SPECIFIC GRAVITY (SG)

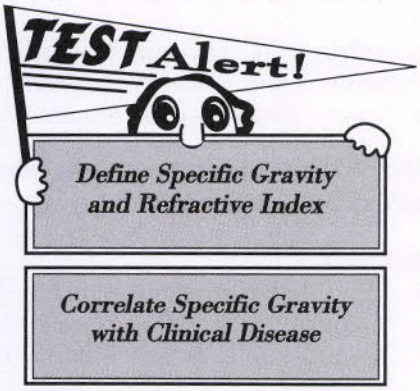
1. Offers the simplest way to check the concentration and dilution function of the kidney tubules
2. Normal urine: 1.002 - 1.035
3. 1.040 is the highest the kidney can concentrate
   a. Higher values are due to either large amounts of glucose or radiographic dyes from renal x-ray procedures
   b. Reagent strip S.G. test not affected by radiographic dyes
4. S.G. is directly proportional to color; the higher the S.G., the deeper the color *(Exception: A pale colored urine with a high S.G. is probably due to glucose -urine is diluted due to loss of concentrating ability by diabetics)*

Diabetes Mellitus or Insipidus?

	Diabetes M.	Diabetes I.
Defect	↓ Insulin	↓ ADH/ AVP
Polyuria	+	+
Polydipsia	+	+
S.G.	↑	↓
Glucose	↑	N
Ketones	↑	N

SPECIFIC GRAVITY MEASUREMENT

METHOD	MEASURED	NOTES
Refractometer - TS	Refractive Index	No temperature corrections Correct for large amount of protein (subtract 0.003 for each gram) and glucose (subtract 0.004 for each gram)
Reagent Strip	Indirect - Colorimetric	pKa change of polyelectrolytes (relative to ionic concentration)



Define Specific Gravity and Refractive Index

Correlate Specific Gravity with Clinical Disease

Chemical Examination

PROTEIN

1. Reagent strip
   a. Principle - "protein error of indicators"
      - ❖ *pH of the protein-testing portion is kept at 3.0*
      - ❖ *Albumin in urine binds to dye*
      - ❖ *Binding shifts the dye's spectrum so that it appears to change color from yellow to green*

   b. More sensitive to albumin than globulin

2. Interfering substances
   a. Highly alkaline urine *(false positive)*

3. Proteinuria
   a. Best single indicator of renal abnormality *(glomerular involvement)*
   b. Associated with multiple myeloma, orthostatic proteinuria *(benign condition resulting in proteinuria after standing)* and strenuous exercise

4. Microalbuminuria
   a. Detected by sensitive albumin tests *(level too low to be detected by routine reagent strip)*
   b. Periodic monitoring benefits patients with diabetes, hypertension, and peripheral vascular disease
   c. Enables patients with low levels of albuminuria to begin treatment before kidney disease occurs
   d. Several commercial methods available for screening

GLUCOSE

1. Reagent strip
   a. Analyzed for diabetes Renal Threshold 160-180 mg/dl
   b. Specific for glucose only
   c. Principle - glucose oxidase *(double sequential enzyme reaction)*
   d. Positive copper reduction test with negative strip = sugar *(or other reducing substance)* other than glucose
   e. Negative copper reduction test with positive strip possible because strip is more sensitive than copper reduction method
   f. Interference by oxidizing agents *(bleach or peroxide)* - false positive

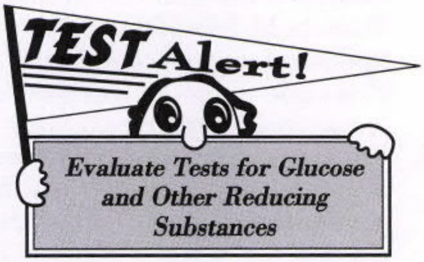
2. Clinitest tablet
   a. For glucose and other reducing sugars
   b. Principle - Benedict's copper reduction test

$CuSO_4$ + NaOH + reducing substance = Cu_2O

- ❖ *Sugars (and other reducing substances) reduce the cupric ion to the cuprous state in the- presence of alkali and heat*
- ❖ *Color of reaction ranges from blue-green to orange-red depending upon amount of sugar*

REAGENT STRIP	COPPER REDUCTION	REASON
0	+	Non-Glucose Sugar or Other Reducing Substances
+	0	Strip More Sensitive to Glucose than Copper Reduction (.1% vs. .2%)

c. **Screening test for galactosemia *(rare congenital carbohydrate metabolic condition in pediatric patients)***
d. **Galactose, lactose, fructose, maltose and pentose give positive results**
e. **Ascorbic acid *(vitamin C)* causes false negative results *(but only in extremely high doses!)***
f. **Use 2 drop method rather than 5 drop method to minimize "pass through" reactions *(reaction goes from negative* [blue] *to positive* [orange] *and back to negative)***


TEST Alert!
Evaluate Tests for Glucose and Other Reducing Substances

KETONES

1. **Acetone, diacetic acid (acetoacetate) and beta-hydroxy-butyric acid - *(end products of fat metabolism)***
2. **Principle *(strip and tablets)* - Sodium nitroprusside + ketone = purple color**
3. **Strip specific for diacetic acid**
4. **Confirmation tests *(Acetest tablets)* specific for diacetic acid and acetone**
5. **Interference by highly pigmented urine and levadopa metabolites cause a false positive result**
6. **Uncontrolled diabetes mellitus, high protein diets and GI disturbances give positive results**

BLOOD

1. **2-step enzymatic procedure**
   a. **Peroxide on strip + blood = O_2**
   O_2 + color producer = color change
2. **Bleach and other oxidizing agents can interfere - false positive results**
3. **Ascorbic acid *(vitamin C)* - false negative results *(newer reagent strips are more resistant to this effect)***
4. **Hemoglobin and myoglobin have peroxidase activity**
5. **Positive results**
   a. **Hematuria associated with systemic bleeding disorders, renal diseases, cystitis, calculi and strenuous exercise**
   b. **Hemoglobinuria associated with hemolytic anemias, incompatible transfusions, malaria and strenuous exercise**
   c. **Myoglobinuria associated with muscle destruction**

BILIRUBIN

1. **Principle *(tablet or strip)* - Diazo Reaction: diazonium salt + bilirubin = bluish purple color**
2. **Ictotest (*tablet*) more sensitive than reagent strip**
3. **False negatives**
   a. **Ascorbic acid (*except newer strips*)**
   b. **Exposure to light**
4. **Bilirubinuria associated with bile duct obstruction and liver damage (*hepatitis and cirrhosis)***

UROBILINOGEN

1. **Principle (*strip*) - Ehrlich's reaction: Para-dimethylaminobenzaldehyde in acid buffer reacts with urobilinogen to produce a peach to pink color**
2. **False negatives**
   a. **Formalin (*preservative*)**
3. **False positives**

   a. Highly pigmented urine
   b. Some medications

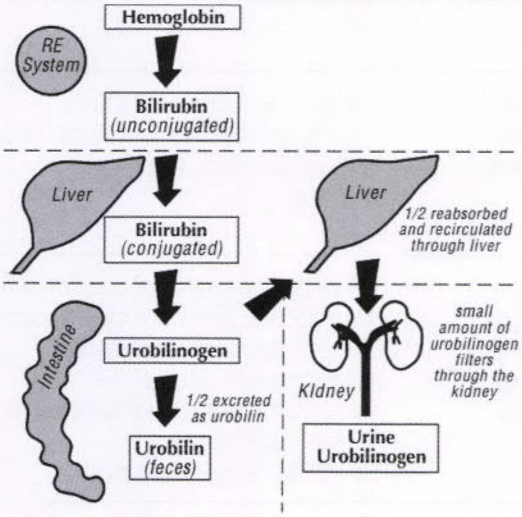
4. Normal: 1 mg/dl or less; 1 EU

5. ↑Urobilinogen
   a. Liver damage (*hepatitis, cirrhosis*)
   b. Hemolytic disease

6. Negative urobilinogen *(not detected on dipstick)* indicates bile duct obstruction

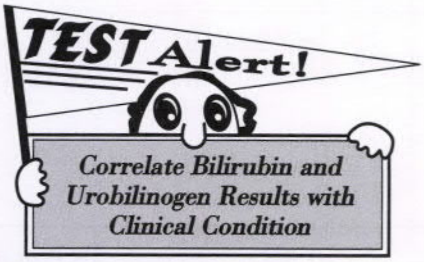
NITRITE (DETECTS NITRATE-REDUCING BACTERIA)

Normal Bilirubin Metabolism


Hemoglobin
RE System
Bilirubin (unconjugated)
Liver
Bilirubin (conjugated)
Liver
1/2 reabsorbed and recirculated through liver
Intestine
Urobilinogen
Kidney
small amount of urobilinogen filters through the kidney
1/2 excreted as urobilin
Urobilin (feces)
Urine Urobilinogen

• Diagram Adapted with Permission from Urodynamics; Siemens Healthcare Diagnostics Inc.; 1979

	BILIRUBIN	UROBILINOGEN
Normal	0	1 EU
Hemolytic	0	↑
Liver Disease	0 or ↑	↑
Obstructive	↑	↓ (N on strip)


TEST Alert!
Correlate Bilirubin and Urobilinogen Results with Clinical Condition

1. Principle (*strip*) Diazo reaction
   a. Nitrite reacts with amine reagent at acidic pH forms diazonium compound
   b. This compound reacts with 3-hyroxy-1,2,3,4 tetrahydrobenz (*h*) -quinolin to produce a pink color

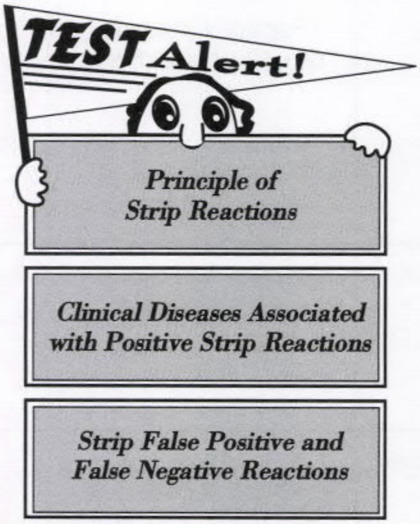
2. False negatives
   a. Lack of dietary nitrate
   b. Urine not in bladder long enough (*4 hr min*) for bacteria to reduce nitrate to nitrite
   c. Bacteria are present but not nitrate reducers
   d. Ascorbic acid (*except newer strips*)

3. Any shade of pink is considered to represent a clinically significant amount of bacteria

LEUKOCYTES

1. Principle (*strip*)
   a. Leukocyte esterase splits an ester to form a pyrrole compound which reacts with a diazo reagent to produce a purple color

2. Positive reactions indicate presence of leukocytes (*pyuria*)


TEST Alert!
Principle of Strip Reactions
Clinical Diseases Associated with Positive Strip Reactions
Strip False Positive and False Negative Reactions

STRIP CORRELATIONS

Positive Test	Correlate Other Tests
pH	Nitrite, Leukocyte esterase, Microscopic
Protein	Blood, Nitrite, Leukocyte esterase, Microscopic
Glucose	Ketone
Ketones	Glucose
Blood	Protein, Microscopic
Bilirubin	Urobilinogen
Urobilinogen	Bilirubin
Nitrite	Protein, Leukocyte esterase, Microscopic
Leukocyte esterase	Protein, Nitrite, Microscopic
Specific Gravity	None

Reagent Strip Reactions

Substance	Reaction Principle	False Positives	False Negatives	Clinical Significance
pH	2 Indicators Provide Wide Spectrum of Color Changes	None	None	Alkaline- may indicate "old" urine;Seen after eating (response to HCl secretion)
Protein	Protein error of indicators- pH of strip=3.0 Dye changes color of strip	Highly alkaline urine	High salt	Proteinuria= Best single indicators of early disease •Glomerular involvement •Can be ↑ after strenuous exercise
Glucose	Glucose oxidase method *(Double sequential enzyme)*	Bleach	Ascorbic acid (newer strips more resistant), Ketones, High S.G. with low pH	Diabetes Mellitus
Ketones	Sodium nitroprusside + ketones= purple color	Highly pigmented urine, Levapoda metabolites	Ascorbic acid (newer strips more resistant), ↑ S.G.	• uncontrolled Diabetes Mellitus • high protein diet (*restricted carbohydrates)* •dehydration *(excess vomiting and diarrhea)*
Blood	Peroxide + Blood= O_2 O_2 + color indicator= color change	Bleach	Ascorbic acid (newer strips more resistant), Ketones, High S.G. with low pH	•hematuria - systemic bleeding disorders, renal disease, cystitis, calculi, strenuous exercise, menstrual contamination •hemoglobinuria- incompatible blood transfusion, malaria, strenuous exercise, hemolytic anemias •myoglobin- muscle destruction
Bilirubin	Diazonium salt+ Bilirubin= bluish/ purple color	Medication color	Ascorbic acid (newer strips more resistant), ↑ S.G., Nitrite	• bile duct obstruction • liver damage *(hepatitis and cirrhosis)*
Urobilinogen	Para-dimethylaminobinzaldehyde + Urobilinogen= peach to pink color *(Ehrlich's reaction)*	Highly pigmented urine, some medications	Nitrite	• liver damage *(hepatitis and cirrhosis)* •*hemolytic anemias*
Nitrite	Nitrite + amine reagent= diazo compound Diazo compound+ 3-Hydroxy-1.2.3.4 Tetrahydrobenz-(h)-quinolin= Pink Color	Medication color	Ascorbic acid (newer strips more resistant),	• Bacteria (*UTI*)
Leukocytes	Leukocyte esterase splits ester to form pyrrole compound. Pyrrole compound +diazo reagent = purple color	Bleach	Glucose, protein, high SG and some antibiotics	• WBC in urine which most likely indicated the presence of bacteria • Reacts with granulocytes not lymphocytes
Specific Gravity	pK_a change of polyelectrolyte	Protein	Alkaline urine	• ↓ Diabetes insipidus (*consistently low*) • Radiopaque dye

Microscopic Examination

STAINS

1. Sternheimer-Malbin - most frequently used supravital stain
2. Sudan III or Oil Red O - confirms the presence of fat or triglyceride

NORMAL URINE SEDIMENT CONSTITUENTS

1. 0-2 rbc/hpf
2. 0-5 wbc/hpf
3. 0-2 hyaline casts/lpf
4. Slight mucus

ABNORMAL URINE CONSTITUENTS

RED CELLS

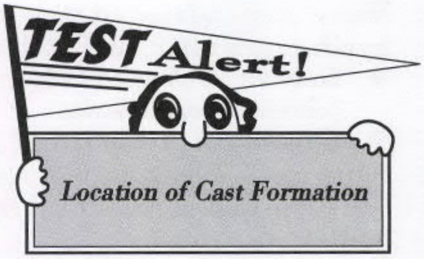
1. May indicate glomerular damage or menstrual contamination
2. May be altered by pH and osmotic pressure to form "ghost", crenated or swollen cells
3. May be confused with yeast cells and oil droplets; add 2% acetic acid to lyse RBC

WHITE CELLS

1. May indicate inflammation or infection (*pyuria*)

CASTS

1. Cylindrical form having parallel sides
2. Formed in the lumen of the distal convoluted tubule and collecting duct



3. Major constituent of casts is uromodulin *(formerly known as Tamm Horsfall protein)*, a glycoprotein secreted by renal tubular epithelial cells
4. Factors that influence cast formation
   a. ↓ pH
   b. ↓ output
   c. ↑ solute concentration (*increased S.G.*)
   d. ↑ protein
5. Types of casts
   a. Hyaline
      - ❖ *Most frequently seen*
      - ❖ *Primarily uromodulin protein*

   b. Red cell cast
      - ❖ *Indicates bleeding from nephron; glomerular dysfunction*
      - ❖ *Solid mass of tightly packed rbc with characteristic orange-red color (unstained sediment)*
      - ❖ *Diagnostic of intrinsic renal disease (glomerulonephritis)*

   c. White cell cast
      - ❖ *Signifies infection or inflammation within the nephron*
      - ❖ *Associated with pyelonephritis*

EPITHELIAL CELLS

EPITHELIAL CELLS	APPEARANCE	NOTES
Squamous	Largest cell Abundant irregular cytoplasm Central nucleus (RBC size)	Least significant Most frequent seen Vaginal lining, lower urethra
Transitional	Round or pear shape Central nucleus Absorb water - Swell to 3X normal size	Renal carcinoma Renal pelvis, bladder, upper urethra
Renal tubular	Most significant Round, eccentric nucleus Larger than WBC	Most significant Tubular necrosis Renal tubules
Oval fat bodies	Highly refractile fat droplets in Renal tubular epithelial cell	Nephrotic syndrome, "Maltese Cross" when polarized, stain with Sudan III or Oil Red O

d. Granular casts
  - *Disintegration of cellular casts*

e. Waxy cast- advanced stage of hyaline, granular, or cellular cast
  - *Indicate prolonged urinary stasis (chronic renal disease)*
  - *Considered "renal failure casts"*

f. Fatty cast - breakdown of epithelial cell casts that contain oval fat bodies

g. Broad casts
  - *Form in collecting ducts that have become dilated (not a good sign!)*
  - *All types of casts can occur as broad casts*

CRYSTALS

1. Formed by the precipitation of urine solutes subjected to changes in pH, temperature and concentration

2. Normal crystals
   a. Acid Urine
     - *Amorphous urates - yellow-brown granules; pink sediment*
     - *Uric acid - variety of shapes, usually a yellow rhomboid form*
     - *Calcium oxalate*
       ☞envelope-shaped
       ☞may be seen in antifreeze poisoning *(young children)*

   b. Alkaline Urine
     - *Amorphous phosphates - white precipitate in urine; microscopically identical to amorphous urates*
     - *Triple phosphate - "coffin lid"*
     - *Ammonium biurate - "thorn apple"*
     - *Calcium carbonate - "dumbbell"*

ABNORMAL CRYSTALS

1. Indicate metabolic disorders or drug metabolites

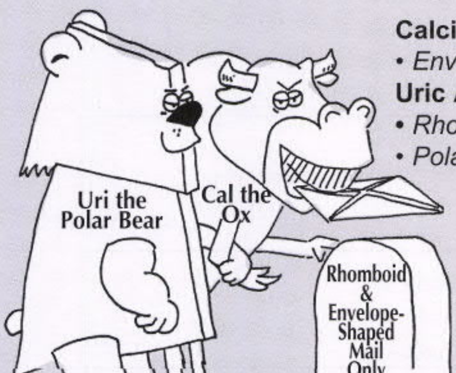
2. Types
   a. Bilirubin - small clusters of fine needles
   b. Cystine - colorless hexagonal plates
   c. Cholesterol - rectangular plates with notched corners
   d. Leucine - yellow-brown spheres with concentric circles or radial striations
   e. Tyrosine - fine, delicate needles (*when seen with leucine crystals, liver disease is indicated)*
   f. Sulfonamide - needles or brown spheres
   g. Radiographic dye - plates
   h. Ampicillin - needles

REMEMBER!

Crystals Seen in "Normal" Urine

Acid Urine

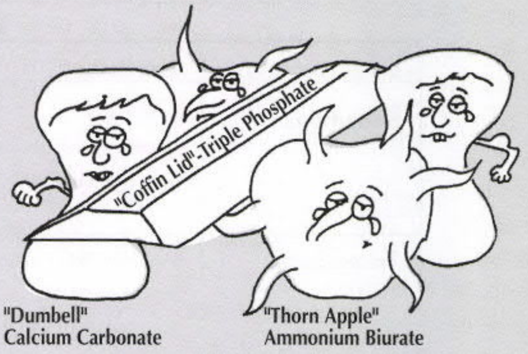
Cal the Ox and Uri the Polar Bear are friends. They hang around Acid Urine.



Calcium Oxalate
- *Envelope Appearance*

Uric Acid
- *Rhomboid shaped*
- *Polarizes*

Alkaline Urine


"Coffin Lid"-Triple Phosphate
"Dumbell" Calcium Carbonate
"Thorn Apple" Ammonium Biurate

The Alkaline Funeral Party

Triple Phosphate
- *Coffin Lid Shaped*

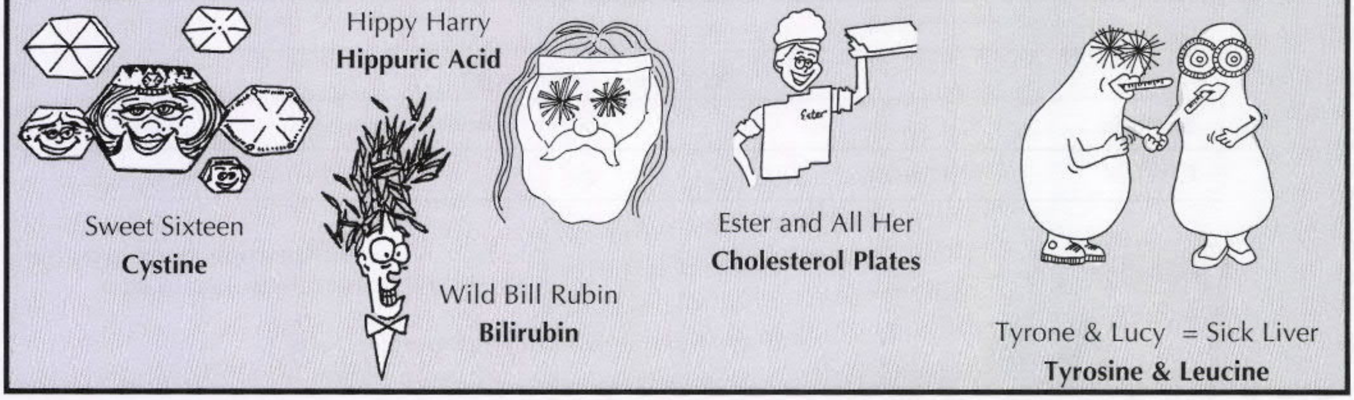
Ammonium Biurate
- *Thorn Apple Crystal*

Calcium Carbonate
- *Dumbell Shaped*

REMEMBER!

Abnormal Crystals

These crazy characters will help you remember abnormal crystal characteristics.



OTHER CONSTITUENTS PRESENT IN URINE

1. Bacteria - rod-shaped *(Bacilli)* most commonly present; correlate with presence/absence of leukocytes
2. Yeast - most often represents a vaginal infection; must correlate with clinical findings
3. Parasites *(Trichomonas vaginalis, Enterobius vermicularis)*
4. Sperm - may be seen in males and females
5. Mucus - no clinical significance
6. Clue cells - squamous epithelial cells with bacteria adhering to them; indicates bacterial vaginosis (*BV*)

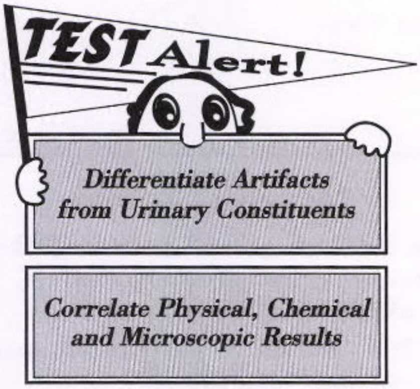
Inclusion Bodies

1. In viral infections, such as Rubella and Herpes RTE cells may contain inclusion bodies.
2. CMV - produces large intranuclear inclusions in RTE
3. Lead poisoning produces cytoplasmic inclusions in RTE
   a. Cytocentrifuge and stain with Papanicolaou stain to visualize
4. Hemosiderin (*a form of iron*) contained in RTE or urine sediment indicates severe intravascular hemolysis, DTR, PCH, or as a result of Hemochromatosis
   a. Cytocentrifuge and stain with Prussian blue stain for iron

ARTIFACTS

ARTIFACTS	CHARACTERISTICS
Powder *(Starch granules from gloves)*	May be confused with crystals Polarized light - Maltese Cross formation, but not round like cholesterol
Fat	Triglyceride stains positive with fat stain Cholesterol does not stain; produces Maltese Cross when polarized
Hair	May be confused with casts
Fiber	Fiber polarizes light - casts do not

MICROSCOPIC CORRELATIONS		
MICROSCOPIC ELEMENT	PHYSICAL CHARACTERISTIC	CHEMICAL CHARACTERISTIC
Red Blood Cell	Turbidity, red color	Blood
White Blood Cell	Turbidity	Protein, Nitrite, Leukocyte esterase
Epithelial Cells	Turbidity	Protein
Casts		Protein
Bacteria	Turbidity	pH, Nitrite, Leukocyte esterase
Crystals	Turbidity	pH


TEST Alert!
Differentiate Artifacts from Urinary Constituents
Correlate Physical, Chemical and Microscopic Results

Pregnancy Testing

1. Human chorionic gonadotropin (*hCG*), a glycoprotein composed of alpha and beta subunits, is secreted by the placenta. The appearance and rapid rise in concentration of hCG in the mother's serum and urine make it an excellent marker for confirming pregnancy.
   a. hCG can be detected as early as 8-10 days after ovulation (*1 day after implantation)*
   b. hCG peaks at 8-10 weeks of pregnancy
   c. First morning specimen preferred *(more concentrated)*

Renal Function Tests

1. Used to test for glomerular filtration and tubular function
2. Tests for glomerular filtration
   a. Clearance tests
   b. B2-Microglobulin
   c. Cystatin C

CREATININE CLEARANCE TEST

1. Most commonly used clearance test to assess GFR *(amount of blood filtered of a particular substance in a given time)*
2. Production and excretion of creatinine is fairly constant from day to day
3. Specimen collection
   a. 24 hour urine
   b. Creatinine > 1 mg/dl measures adequacy of collection
4. Mathematical formula:

$$\text{Creatinine Clearance} = \frac{U \times V}{P}$$

U = Concentration of urine creatinine mg/dL
V = Volume of urine in ml/time in minutes
P = Concentration of plasma creatinine mg/dL

5. Normal Creatinine Clearance = 120 ml/min for adults; ↓ with age
6. Correction for Kidney Mass *(Kidney Mass Proportional to Body Size)*

$$\text{Clearance} = \frac{U \times V}{P} \times \frac{1.73}{A}$$

A = Body Surface Area

7. Example:

Plasma Creatinine = 1.5 mg/dL
Urine Creatinine = 120 mg/dL
Urine Volume = 1.2 L
Surface Area = 1.30 mm^3
Collection Time = 24 hours

$$\text{Volume} = \frac{1200\text{ ml}}{1440\text{ min}} = 0.833$$

$$\frac{120\text{ mg/dL} \times (0.833\text{ ml/min})}{1.5\text{ mg/dL}} = 66.67\text{ ml/min}$$

$$\frac{66.67 \times 1.73}{1.30} = 88.71\text{ ml/min}$$

ESTIMATED GRF

1. Assists in detecting chronic kidney disease
2. More sensitive than creatinine clearance
3. Calculation based on serum creatinine, patient's age, gender, and ethnicity

B2-MICROGLOBULIN

1. Useful marker of renal tubular function.
2. ↑ plasma concentrations indicate a reduced GFR *(not specific)*

CYSTATIN C

1. May provide an equal or better detection of adverse changes in GFR
2. Disadvantages:
   a. Higher cost than creatinine clearance
   b. Possible variable results among individuals

Renal Diseases

Findings in Renal Disease

DISEASE	LAB FINDINGS
Acute Glomerulonephritis	Gross hematuria, Smoky Turbidity, RBC Casts, Waxy
Chronic Glomerulonephritis	Hematuria; All Types of Casts, but Only Occ to Few RBC Casts
Acute Pyelonephritis	Turbid, Pos Nitrite, Pos Leukocyte Esterase, WBC Casts, Bacteria
Chronic Pyelonephritis	Pos Nitrite, Pos Leukocyte Esterase; All Types of Casts, but Only Occ to Few WBC Casts
Nephrotic Syndrome	May See "Free" Fat Droplets; Fatty Casts; Oval Fat Bodies
Cystitis/Lower Urinary Tract Infection	Bacteria; WBC's

Body Fluids

CEREBROSPINAL FLUID (CSF)

1. Reasons for analysis:
   a. Meningitis
   b. Encephalitis
   c. Syphilis
   d. Brain abcess / Tumor
   e. Intracranial hemorrhage
   f. Leukemia / Lymphoma with CNS involvement
2. Normally clear, colorless
3. Xanthochromia - pink, orange, or yellow CSF supernatant *(usually due to hemoglobin)*
4. Important to differentiate between intracranial hemorrhage and traumatic collection

DISTRIBUTION OF CSF TUBES

	LABORATORY SECTION(S)	POSSIBLE TESTS
Tube #1	Chemistry and/or Serology	Protein, Glucose, Lactate, VDRL, Latex agglutination tests
Tube #2	Microbiology	Gram stain, culture, India ink
Tube #3	Hematology	Cell count and differential

DIFFERENTIATION BETWEEN HEMORRHAGE VS. TRAUMATIC TAP

	HEMORRHAGE	TRAUMATIC TAP
Appearance	All tubes equally red (bloody)	Subsequent clearing of blood in each tube
Supernatant	Xanthochromic	Clear
Presence of Clots	No	Yes, due to fibrinogen

Differentiating Causes of Meningitis - CSF Studies

	PROTEIN	GLUCOSE	WBC POPULATION	LACTATE
Bacterial	↑↑	↓	Neutrophils	↑
Viral	↑	N	Lymphocytes	N
Fungal	↑	N - ↓	Lymphocytes and Monocytes	↑

Differentiate Traumatic Tap from Intracranial Hemmorrhage

5. Normal Analyte Values
   a. Protein - 15-45 mg/dl
   b. Glucose - 60-70% plasma glucose
   c. Cell Count - 0-5 WBCs/microliter(*ul*)
   d. Differential - 70% lymphocytes; 30% monocytes

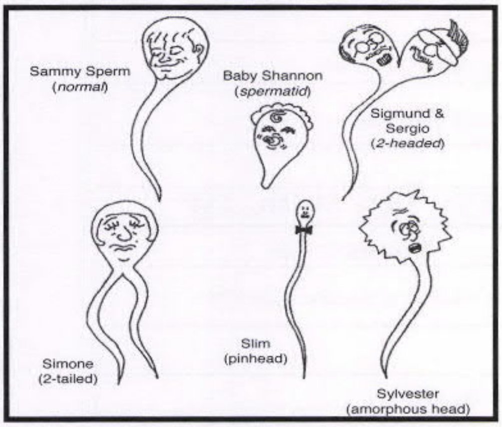
6. CSF Electropheresis - oligoclonal banding = multiple sclerosis (↓ *glucose and* ↑ *protein)*

MANUAL CELL CALCULATION FOR BODY FLUIDS

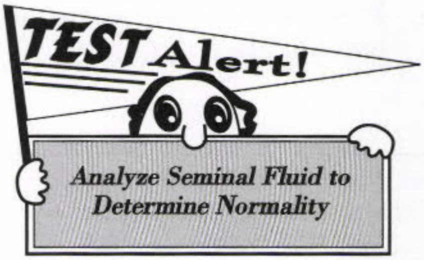
$$\frac{\text{\# Cells Counted X Dilution}}{\text{\# Squares Counted X Depth } \textit{(0.1)}}$$

SEMINAL FLUID

1. Reasons for semen analysis
   a. Infertility (most common)
   b. Post vasectomy
   c. Forensic medicine — presence of acid phosphatase confirms presence of semen in alleged rape cases; flavin in semen fluoresces on clothing under UV light



2. Reference ranges
   a. Volume - 2-5 ml
   b. Count - 20-250 million/ml
   c. Motility - > 50%
   d. Morphology - < 30% Abnormal Forms
   e. Viability > 75% live forms
      ❖ *Azoospermia - no sperm*
      ❖ *Oligospermia - < 20 million/ml*



Analyze Seminal Fluid to Determine Normality

SEROUS FLUIDS

1. Reasons for analysis:
   a. Sepsis
   b. Malignancy
   c. Systemic disease

SYNOVIAL FLUID

1. Reasons for analysis:
   a. Sepsis
   b. Hemorrhage
   c. Crystal induced inflammation

2. Normal appearance: clear, pale yellow

3. Monosodium urate crystals = gout

4. Calcium pyrophosphate crystals = pseudogout

5. Compensated polarization:
   a. Calcium pyrophosphate appears blue when parallel to compensator and yellow when perpendicular
   b. Monosodium urate appears yellow when parallel to compensator and blue when perpendicular

6. Cell count normal ranges:
   a. < 200 WBC/μL
   b. < 2000 RBC/μL

Transudates vs. Exudates

	TRANSUDATE	EXUDATE
Color	Colorless	Yellow-White, (inflammation); Red-Brown (hemorrhage); Yellow-Brown (bilirubin); Milky-Green (chylous fluid)
Turbidity	Clear, Watery	Cloudy, Viscous
Specific Gravity	< 1.015	> 1.015
Protein	< 3 g/dl	> 3 g/dl
LD (Lactic Dehydrogenase)	< 200 IU	> 200 IU
Cell Count	< 1000/µl	> 1000/µl
↑ Associated With	Congestive Heart Failure, Changes in Hydrostatic Pressure	Infections and Malignancies

PLEURAL PERICARDIAL AND PERITONEAL FLUID

1. Effusion — build-up of fluid in a body cavity due to a pathologic process

2. Cell count and differential performed in same manner as CSF

AMNIOTIC FLUID

1. Reasons for analysis
   a. Fetal well-being
   b. Fetal lung maturity

2. Fetal lung maturity tests
   a. Lecithin/Sphingomyelin (*L/S*) ratio
      ❖ *Measures the phospholipids lecithin and sphingomyelin to assess fetal lung maturity*
      ❖ *If the L/S ratio is > 2.0, fetal lungs are usually mature (diabetic mothers — PG must be present for FLM)*
   b. Phosphatidylglycerol (*PG*)
      ❖ *Lipid component of pulmonary surfactants*
      ❖ *Not usually detected until 35 weeks of gestation*
      ❖ *Absence does not rule out mature fetal lungs*
   c. Lamellar body counts
      ❖ *Secreted into alveolar lumen at 20-24 weeks of gestation*
      ❖ *Amniotic fluid is analyzed on automated instrument by using the platelet count value*
      ❖ *Advantages*
         ☞ *small sample volume*
         ☞ short turnaround time
         ☞ low cost
         ☞ easily interpreted

3. Other amniotic fluid testing
   a. Alpha-fetoprotein (*AFP*)
      ❖ ↑ *associated with neural tube disorders (such as Spina Bifida)*
   b. Bilirubin
      ❖ *Reliable estimate of fetal red cell destruction due to maternal antibody*
   c. Foam stability index
   d. Fluorescence Polarization Assay

SWEAT

1. ↑ sweat electrolytes, sodium and chloride, confirms the diagnosis of cystic fibrosis

DETECTION OF MALIGNANCY

1. Benign tissue cells must be differentiated from malignant cells in body fluids.

DIFFERENTIATING BENIGN AND MALIGNANT CELLS

BENIGN CELLS	MALIGNANT CELLS
Distinguishable cell borders in clumps	Poorly defined
Flat clusters - not ball-like	Ball-like spherical clusters
Individual cells	Often cells within cells (cannibalism)
May be large, but not giant, tend to be uniform	Often bizarre, monstrous - non-uniform
N/C ratio low	Frequently high N/C ratio
Smooth, clear membrane	Irregular nuclear membrane
Even chromatin	Uneven chromatin
Can be multinucleated but nuclei uniform	Multinucleated forms with varied nuclear sizes
Nulei round or oval	Nuclear molding (mosaic)
Nucleus even, no clefts	Nuclear clefts
May be vacuolated	May be vacuolated with vacuoles over nucleus

Fluids with cells suspicious for malignancy always should be referred to cytology and for pathologist's review.

The cells should be reported as atypical and suspicious for malignancy on the differential cell report.

Summary of Body Fluids

FLUID	SOURCE	APPEARANCE	LAB TESTS	CLINICAL SIGNIFICANCE
Serous	*Ultrafiltrate of plasma; fills organ cavities*	*Normal; clear and pale yellow*		
PLEURAL	Thoracic Cavity *(around lungs)*	Turbid - white cells, bacteria Bloody - trauma, malignancy Milky - chylous fluid	Cell Counts:	↑ Red Cells - trauma, malignancy ↑ Neutrophils - bacteria ↑ Lymphocytes - tuberculosis, malignancy
			Glucose:	↓ Tuberculosis, rheumatoid inflammation, malignancy
			pH:	↓ Tuberculosis, malignancy, esophageal rupture
			Amylase:	↑ Pancreatitis
			CEA *(carcinoembryonic antigen)*:	↑ Malignancy
PERICARDIAL	Percardial Cavity *(around heart)*	Turbid - infection, malignancy Bloody - tuberculosis, tumor, cardiac puncture Milky - lymphatic drainage	Cell Counts:	↑ Red Cells - tuberculosis, tumor, cardiac puncture ↑ Neutrophils - bacterial endocarditis
			Glucose:	↓ Bacterial infection
			CEA:	↑ Malignancy
PERITONEAL *(ascites)*	Peritoneal Cavity *(around abdomen)*	Turbid - peritonitis, cirrhosis Bloody - trauma Milky - chylous fluid Green - bile	Cell Counts:	↑ Red Cells - trauma ↑ Neutrophils - peritonitis
			Glucose:	↓ Tubercular peritonitis, malignancy
			Amylase:	↑ Pancreatitis, GI perforation
			Alkaline Phosphatase:	↑ Intestinal perforation
			Urea/Creatinine:	↑ Ruptured bladder
Other Fluids			*Other Fluids*	*Other Fluids*
SWEAT	Sweat Glands *(of skin)*	Clear and colorless	Sodium Chloride *(sweat test)*:	↑ in Cystic Fibrosis
SYNOVIAL	Synovial Membrane *(around joints)*	Clear, pale yellow, and viscous Bloody - hemorrhagic arthritis Milky - crystals, cells Green tinge - bacteria Deep yellow - inflammation	Cell Counts:	↑ Red Cells - hemorrhage ↑ Neutrophils *(> 25%)* - sepsis ↑ Lymphocytes - non-septic inflammation
			Crystals:	Uric Acid *(monosodium urate)* - gout
			Ropes Clot Test:	↓ clot - arthritis
GASTRIC	HCl and Pepsin *(secreted in stomach)*	Depends on diet	Titratable Acidity:	↓ in pernicious anemia *(no intrinsic factor)* ↑ in duodenal ulcer, Zollinger-Ellison Syndrome
AMNIOTIC	Amniotic Sac	Clear and colorless - normal Yellow-greeen - bilirubin, red cell destruction	Bilirubin:	↑ in HDN *(indicates red cell destruction)*
			L/S Ratio:	*>2.0* = fetal lung maturity
			Phosphatidylglycerol:	↑ indicates fetal maturity *(similar to L/S ratio)*
			Creatinine:	Fetal age
			Alpha Fetoprotein:	↑ indicates neural tube disorders

URINALYSIS/BODY FLUIDS SAMPLE QUESTIONS

1. Which of the following changes occur when a urine specimen is left at room temperature for longer than 1-2 hours?
   - A. Ketones ↓
   - B. Bilirubin ↑
   - C. Bacteria ↓
   - D. Glucose ↑

2. A urinalysis on a 3 year old revealed a positive copper reduction test and a negative glucose oxidase test. How would these results be interpreted?
   - A. A strong oxidizing agent is present.
   - B. Copper reduction tests are more sensitive than glucose oxidase tests.
   - C. Galactose is present.
   - D. Glucose and lactose are both present.

3. Chemical and microscopic analysis of a urine specimen yields the following results

   Protein: 4+
   RBC 0-2 /hpf
   WBC 0-5 / hpf
   Few hyaline casts
   Moderate fatty, waxy, and granular casts
   Many oval fat bodies

   These results are consistent with which diagnosis?
   - A. Hepatic insufficiency
   - B. Glomerulonephritis
   - C. Nephrotic syndrome
   - D. Pyelonephritis

4. An abnormally high specific gravity may be seen in which of the following conditions?
   - A. Chronic renal disease
   - B. Diabetes insipidus
   - C. Metabolic acidosis
   - D. Radiographic dye injection

5. The reagent strip reaction used to test for the presence of glucose is based on the principle of
   - A. A buffered reaction of mixed enzyme indicators.
   - B. Acid precipitation of glucose salts.
   - C. Double sequential enzyme reactions.
   - D. Glucose producing a change in pH in a buffered system.

6. An elevated urine urobilinogen and a negative test for urine bilirubin may indicate which of the following conditions?
   - A. Acute hepatic toxicity
   - B. Biliary obstruction
   - C. Hemolytic disease
   - D. Urinary tract infection

7. Calculate the creatinine clearance of a 24-hour urine specimen using the following data:

Urine creatinine	500 mg/L
Plasma creatinine	8 mg/L
Urine volume	1.5 L

   - A. 6.5 ml/min
   - B. 0.75 ml/24 hours
   - C. 65 ml/min
   - D. 85 ml/min

8. An L/S ratio of 2.7 indicates
   - A. Fetal distress.
   - B. Fetal lung maturity.
   - C. Fetal red cell destruction.
   - D. Inadequate fetal pulmonary surfactants.

9. Cerebrospinal fluid results reveal an ↑ protein, normal glucose, normal lactate and ↑ lymphocytes. These results most likely indicate
   - A. Bacterial meningitis.
   - B. Fungal meningitis.
   - C. Multiple Sclerosis.
   - D. Viral meningitis.

ANSWERS AND RATIONALE

1. A

When a specimen is left unpreserved at room temperature, several changes take place. Option A is correct because ketones will ↓ due to bacterial metabolism of acetoacetate to acetone. Option B is incorrect because bilirubin ↓ as it is photooxidized to biliverdin. Option C is incorrect because bacteria multiply causing an ↑ result. Option D is incorrect because glucose ↓ as bacteria utilize the glucose for glycolysis.

2. C

The copper reduction method will detect any reducing sugar, whereas, the glucose oxidase method is specific for glucose. Option A is incorrect because oxidizing agents give false positive results with the glucose oxidase method and not the copper reduction method. Option B is incorrect because the glucose oxidase method detects minimum glucose levels of 100 mg/dl, while the copper reduction method only detects minimum levels of 200 mg/dl. Option D is incorrect because if these both sugars were present, both tests would be positive.

3. C

Hallmarks of the nephrotic syndrome include fatty casts, oval fat bodies, and markedly increased protein in the urine. Option A does not usually demonstrate cast formation. Option B would show a positive blood result and many red cell casts. Option D would have many WBC's and white cell casts.

4. D

Radiographic dyes are water soluble substances that are readily excreted in urine that cause a significantly increased specific gravity. In Option A, the kidneys are no longer able to concentrate or dilute urine causing a fixed specific gravity usually around 1.010. Option B is incorrect because persons with diabetes insipidus are unable to produce concentrated urine due to defective ADH production. In Option C, the body reacts to maintain acid-base balance by inducing the kidneys to eliminate H+ ions through the urine which does not affect the specific gravity.

5. C

The reagent strip reaction combines two separate reactions catalyzed by two different enzymes. Glucose is combined with oxygen in the presence of the enzyme glucose oxidase. Gluconic acid and hydrogen peroxide are formed from this reaction. Hydrogen peroxide formed in the first reaction oxidizes a chromogen in the presence of the enzyme peroxidase to produce a color change. Thus, this method is termed a double sequential enzyme reaction.

6. C

Increased hemoglobin degradation associated with hemolytic conditions such as transfusion reactions and sickle cell disease results in the production of large amounts of unconjugated bilirubin which is presented to the liver to be conjugated. This large amount of bilirubin then goes to the intestine and is broken down by bacteria into urobilinogen. Because larger amounts of bilirubin are excreted into the intestine and larger amounts of urobilinogen are made, increased amounts of urobilinogen are reabsorbed into the circulation. As a result, the urinary bilirubin remains negative but the urinary urobilinogen increases above its normal level. Option A would result in the production of increased levels of bilirubin and urobilinogen due to liver dysfunction. Option B is incorrect because bilirubin, due to the obstruction, could not be converted to urobilinogen. Less than normal amounts of urobilinogen would be excreted in the kidney. (*The dipstick will show normal urobilinogen because it cannot detect lower than normal amounts.*) Option D does not affect bilirubin metabolism.

7. C

To calculate the creatinine clearance:

$$\frac{U \times V}{p}$$

Where:

U= the concentration of urine creatinine in mg/ml
V= the volume of urine in ml/min
p= the concentration of plasma creatinine in mg/ml

The first step is to convert the results to the correct units. Because the volume of urine must be in ml/min, convert the volume of urine to ml then divide the ml by 1440 (*number of minutes in 24 hours*). In this case, 1.5 L = 1500 ml = 1.04 ml/min. If the height and weight of the patient are known a correction for the kidney mass is calculated. Use this formula:

$$\frac{U \times V}{p} \times \frac{1.73}{A}$$

Where: A= the surface area of the body.

The surface area of the body can be determined by a nomogram which can be found in several reference books.

$$= \frac{500\text{ mg/L} \times 1.04\text{ mL/min}}{8\text{ mg/L}}$$

$$= \frac{520\text{ mL/min}}{8}$$

$$= \underline{65.1\text{ mL/min}}$$

8. B

Lecithin and sphingomyelin are produced by the fetal pulmonary system in a relatively constant rate until the 35th week of gestation, at which time the concentration of sphingomyelin decreases and the concentration of lecithin increases in the amniotic fluid. Before this time, the L/S ratio is usually less than 2.0 which is associated with immaturity of the fetal lungs. When the L/S ratio reaches 2.0 or greater it is indicative of fetal lung maturity.

9. D

These results are indicative of viral meningitis. Option A would show an ↑ in neutrophils. Option B would show an ↑ lactate and possible monocytes. Option C would show a ↓ glucose and oligoclonal banding with electrophoresis.

☞ Be able to identify cells, casts, bacteria and yeast from graphic images. Correlate microscopic findings with chemical tests and disease states.

CHEMISTRY

by Larry Broussard and Mary Muslow *

Sample Collection and Handling

1. Serum (*red top*) for most chemistry tests
2. Plasma — Heparin better than oxalate, EDTA, citrate
3. Heparin (*on ice*) - ammonia, blood gases, lactic acid
4. Sodium fluoride (*gray top*)
   - a. Glucose (*slows glycolysis*)
   - b. NOT for BUN *(inhibits urease)*
5. Acid Phosphatase must be stabilized or ↓ pH
6. EDTA
   - a. NOT for Na^+ or K^+ (*EDTA contains* Na^+ *and* K^+)
   - b. Not for Ca^{++} since calcium is chelated in EDTA (*will cause a false* ↓)

REMEMBER!

Hemolysis . . .

as the cell bursts, help (c)KLAMP the leak!

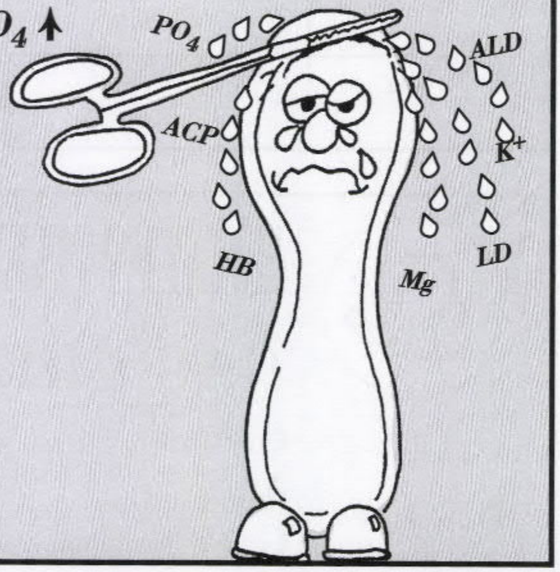
K *K^+ ↑*

L *LD ↑*

A *Aldolase (ALD ↑)*
Acid Phosphatase (ACP ↑)

M *Mg^{++} ↑*

P *PO_4 ↑*



↑ Hemoglobin =

- ***Protein Electrophoresis May Show Extra Band***
- ***↑ Fe^{++}***

But, Bilirubin ↓ or ↑ (Depending on hemoglobin and bilirubin concentrations)

Carbohydrates : Glucose

1. Digestion, metabolism, regulation
   - a. End product of carbohydrate digestion in the intestine
   - b. Blood glucose is maintained at a fairly constant level by hormones *(Insulin ↓; all others ↑; see table on page 102)*
   - c. Provides energy for life processes

TEST Alert!

Relationship Between CSF and Plasma Glucose Levels

2. Specimen collection and handling
   - a. Glucose levels decrease approximately 10 mg/dL per hour in whole blood *(7%)*
   - b. Refrigerated serum is fairly stable
   - c. Sodium fluoride *(anticoagulant)* slows glycolysis *(gray top tube)*
   - d. Fasting reference range for serum or plasma is 70-99 mg/dL
   - e. Arterial and capillary values are 2-3 mg/dL higher
   - f. "Normal" CSF values are two-thirds *(approximately 60-65%)* of plasma levels

---

*Original contribution by Melanie S. Chapman

HORMONAL ACTIVITY AFFECTING SERUM GLUCOSE LEVELS

HORMONE	SOURCE	ACTION
Insulin	beta cells of Islets of Langerhens of pancreas	• ↓ serum glucose • Stimulates glucose uptake by cells
Glucagon	alpha cells of pancreas	• ↑ glucose • Stimulates glycogenolysis *(breakdown of glycogen → glucose)*
ACTH	anterior pituitary	• ↑ glucose • Insulin antagonist
Growth Hormone	anterior pituitary	• ↑ glucose • Insulin antagonist • Acromegaly = hyperglycemia
Cortisol	adrenal cortex	• ↑ glucose • Stimulates gluconeogenesis (*glucose from non-carbohydrate sources*)
Human Placental Lactogen	placenta	• ↑ glucose • Insulin antagonist
Epinephrine	adrenal medulla	• ↑ glucose • Stimulates glycogenolysis • Pheochromocytoma-tumor of adrenal medulla → hyperglycemia
T_3 & T_4	thyroid gland	• ↑ glucose • Stimulates glycogenolysis

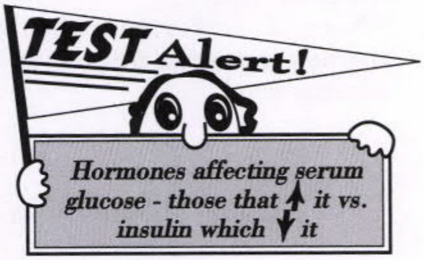
REMEMBER!

GAG CHET

Glucagon
ACTH
Growth Hormone

Cortisol
Human Placental Lactogen
Epinephrine
T$_3$ & T$_4$

All cause an increase in serum glucose



Hormones affecting serum glucose - those that ↑ it vs. insulin which ↓ it

REMEMBER!

Fido the Diabetic Dog

Cause: ↓ or No Insulin Production

SYMPTOMS:
- Tired
- Polyuria
- ↑ Thirst & Hunger
- Weight Loss
- Poor Wound Healing

LAB TESTS:
- ↑ Glucose (*serum & urine*), ↑ A1C
- May Have ↑ Cholesterol
- Diabetic Acidosis:
  ↓ Na^+
  ↑ K^+
  ↓ Cl^-
  + Ketones (*blood & urine*)

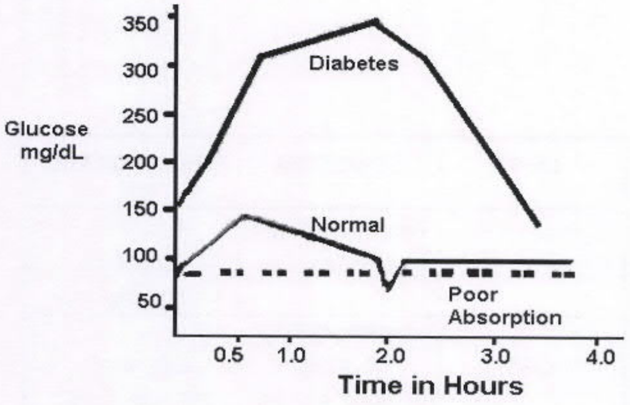
TEST INTERPRETATION TO DIAGNOSE DIABETES MELLITUS

STAGE	TEST			
	Fasting plasma glucose (FPG)	**Casual plasma glucose**	**Oral glucose tolerance test (OGTT) ***	**A1c**
Normal	FPG <100 mg/dL		2 hr PG <140 mg/dL	≤ 5.6%
Diabetes Mellitus	FPG ≥126 mg/dL	≥200 mg/dL plus unexpected weight loss, polyuria, polydipsia	2 hr PG ≥200 mg/dL	≥ 6.5%
Pre-diabetes	Impaired Fasting Glucose (IFG) FPG ≥100 <126 mg/dL		Impaired Glucose Tolerance (IGT) 2 hr PG ≥140 <200 mg/dL	5.7 - 6.4%

* Oral Glucose Tolerance Test (OGTT) Dose:
Standard: 75 g • Children: 1.75 g/kg up to 75 g
Pregnancy (for Gestational Diabetes) - 50 g screen, if 1 hr glucose ≥140 mg/dL, then give 100 g dose and compare fasting, 1, 2, 3 hr levels to acceptable limits

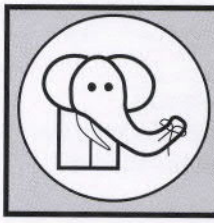
TESTS TO MONITOR DIABETES	NOTES
A1c or HbA1c Glycosylated or Glycated Hemoglobin	• Glucose Attaches to Hemoglobin • Average Glucose Level Over 90 Days (1-3 Months) • Express as % and Convert to eAG (eABG) • Diagnosis of Diabetes • Inaccurate if Abnormal Hemoglobin Pattern
Fructosamine	• Assess Intermediate-Term Control • Glucose Attaches to Proteins Including Albumin • Average Glucose level Over 2-3 Weeks
Urinary Albumin (formerly called microalbumin)	• Detects Small Amounts of Albumin in Urine to Assess Early Renal Damage
C Peptide	• Proinsulin Cleaved to Give C Peptide and Insulin • Reflects Endogenous Insulin Production if Patient is Taking Insulin

Glucose Tolerance Curve



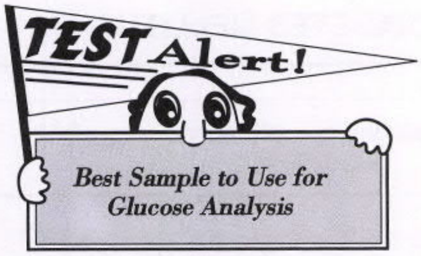
estimated Average Glucose (eAG)

1. ADA recommends reporting eAG when A1c is reported
2. Formula:
   eAG = (28.7 X A1c) - 46.7
3. Example: A1c = 6%
   eAG = (28.7 X 6) - 46.7
   eAG = 126
4. For every 1% change in A1c there is approximately 28 mg/dl change in eAG



REMEMBER!

A1c of 6%= eAG of 126 mg/dl add 28 mg/dl for every 1% increase in A1c



Best Sample to Use for Glucose Analysis

Evaluate Laboratory Results to Diagnose and Assess Diabetic Control

5. Glucose Methods : Enzymatic
    a. Glucose oxidase (*Glu. Ox.*)
        ❖ *Coupled Reactions*

Glu. Ox. converts glucose to gluconic acid + H_2O_2

Peroxidase converts H_2O_2 to O_2

O_2 + chromogenic acceptor gives color

❖ *Method used on urine dipsticks*
❖ *Modified method measures oxygen consumption with a pO_2 electrode*
❖ *Interfering substances - extremely high doses of ascorbic acid (vitamin C) - cause ↓ results*

    b. Hexokinase
        ❖ *Reference method*
        ❖ *Coupled Reactions*

$$\text{Glucose} + \text{ATP} \xrightarrow{\text{Hexokinase}} \text{G6P} + \text{ADP}$$

$$\text{G6P} + \text{NADP} \xrightarrow{\text{G6PD}} \text{NADPH}$$

NADPH measured directly at 340 nm or coupled to chromogen and measured in visible range

Carbohydrate Tests Other than Glucose

1. Lactose tolerance
    a. Evaluate deficiency of lactase in small bowel
    b. Ingest lactose and measure blood glucose to assess absorption and conversion to glucose
    c. May develop transient lactose intolerance when recovering from diarrhea or intestinal infection — avoid milk products until recovered
2. Xylose absorption
    a. Xylose absorbed and excreted into urine without need of pancreatic enzymes
    b. Helps distinguish intestinal malabsorption *(see low absorption of xylose)* and pancreatic-based malabsorption *(see normal absorption of xylose)*
3. Lactic Acid (*Lactate*)
    a. Intermediate in carbohydrate metabolism
    b. Indicator of oxygen deprivation *(exercise, drugs, diseases, etc.)*
    c. Build-up results in lactic acidosis
    d. Sample collection and handling critical
        ❖ *Glycolysis increases lactic acid levels*
        ❖ *No exercise before collection*
        ❖ *Separate plasma and refrigerate*

Lipids and Lipoproteins

CHEMISTRY AND PHYSIOLOGY

1. Organic substances insoluble in water, soluble in organic solvents

2. Human plasma lipids are cholesterol, triglycerides, phospholipids and non-esterified fatty acids

3. Transported in the form of lipoproteins (*lipids combine with proteins in the liver to form lipoproteins*)
    a. 60-70% by low density lipoproteins (*LDL*)
    b. 20-35% by high density lipoproteins (*HDL*)
    c. 5-12% by very low density lipoproteins (*VLDL*)

4. Apolipoproteins
    a. Protein portion of lipoproteins
    b. Apolipoprotein A is the major protein of HDL
    c. Apolipoprotein B (B100 or B48) is present in all atherogenic lipoproteins

LIPID	FUNCTION	TRANSPORTED BY:
Triglycerides	Primary form of lipid storage	chylomicrons (exogenous) VLDL (endogenous)
Cholesterol	Important in cellular physiology Precursor to steroid hormones	LDL - to cells HDL - out of cells

LIPOPROTEIN CLASSES

1. **4 major classes based on particle size, chemical composition, flotation characteristics and electrophoretic mobility; more protein higher density; more lipid, lower density**

LIPOPROTEIN CLASSES	
CLASS	**TRANSPORTED LIPIDS**
Chylomicrons	exogenous (dietary) triglycerides
VLDL	atherogenic; endogenous triglycerides
LDL	atherogenic; cholesterol to cells (heart)
HDL	atheroprotective; cholesterol out of cells

> **REMEMBER!**
>
> **Lipoproteins**
>
> - HDL is helpful (*takes cholesterol from arterial wall)* Hooray Alpha!
> - LDL is lethal (*brings cholesterol to arterial wall*) Boo! Hiss! Bad Beta
> - VLDL *(longest initials*) = pre-beta & endogenous triglycerides (*most letters*)

SPECIMEN COLLECTION AND HANDLING

1. **12 hour fast required**
2. **Non fasting may be cause of**
   a. **Turbid serum with layer of chylomicrons following refrigeration (*abnormal finding in fasting specimens*)**
   b. **↑ triglycerides**

LIPID METHODS

1. **Cholesterol**
   a. **Enzymatic methods *(cholesterol oxidase)* have replaced colorimetric methods**
2. **Triglycerides**
   a. **Enzymatic methods best**
      - ***Involve liberation of glycerol by lipase***
      - ***Glycerol contamination from stoppers of evacuation tubes or ingestion of glycerol-coated medication can cause falsely ↑ results***
3. **HDL-cholesterol (HDLc)**
   a. **Direct or homogenous assays — measure HDLc without pretreatment**
   b. **Indirect — remove chylomicrons, VLDL, and LDL by precipitation; analyze supernatant for HDLc**
4. **LDL-cholesterol (LDLc)**
   a. **Direct or homogenous assays — measure LDLc without pretreatment**
5. **Electrophoresis**
6. **Ultracentrifugation**
   a. **Reference method**
   b. **Separates lipoproteins by their rates of flotation using ultracentrifugation**
7. **Friedewald method for the calculation of LDLc and VLDLc — cannot use when triglycerides (TG) > 400 mg/dL**

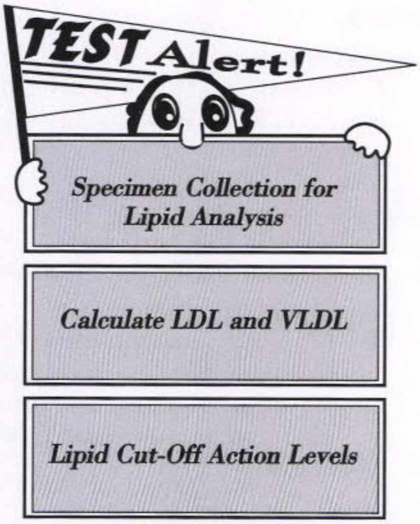
$$\text{LDLc} = \text{TC}-(\text{HDLc}) - \frac{\text{TG}}{5}$$

TC = Total Cholesterol

$$\text{VLDLc} = \frac{\text{TG}}{5}$$

LDL-CHOLESTEROL (LDLc) DECISION LEVELS AND RECOMMENDED TREATMENT			
LDLc ACTION LEVEL	**RISK CATEGORY**	**LDLc GOAL**	**TREATMENT**
≥ 190 mg/dL	no CHD; < 2 risk factors	< 160 mg/dL	Drug
≥ 160 mg/dL	no CHD; ≥ 2 risk factors	< 130 mg/dL	Drug
≥ 160 mg/dL	no CHD; < 2 risk factors	< 160 mg/dL	Diet
≥ 130 mg/dL	no CHD; ≥ 2 risk factors	< 130 mg/dL	Diet
≥ 130 mg/dL	CHD	≤ 100 mg/dL	Drug
> 100 mg/dL	CHD	≤ 100 mg/dL	Diet

from National Cholesterol Education Program



Specimen Collection for Lipid Analysis

Calculate LDL and VLDL

Lipid Cut-Off Action Levels

Cardiac Risk Factors

1. Positive Risk Factors
   a. Age >45 years men, >55 years women
   b. Family history of premature CHD
   c. Current cigarette smoking
   d. Hypertension systolic bp >120
   e. HDLc <40 mg/dl
   f. Diabetes Mellitus
   g. Metabolic Syndrome

2. Negative Risk Factors
   a. HDLc > 60 mg/dl

TREATMENT GUIDELINES

1. Lipid Screening
   a. Can be nonfasting sample
   b. Goals
      - ❖ *Total Cholesterol < 200 mg/dL*
      - ❖ *HDLc > 35 mg/dL*
      - ❖ *Triglycerides < 150 mg/dL*
      - ❖ *LDLc < 100 mg/dl*
   c. Follow-up testing if levels outside goals
      - ❖ *12 hour fasting sample*
      - ❖ *Lipoprotein analysis*
   d. Begin treatment based on LDLc results (*see page 105*) and other risk factors (*see above*)

Amino Acids

GENERAL

1. Aminoacidurias
   a. Overflow-plasma level above renal threshold as result of metabolic disorder
      - ❖ *PKU — enzyme deficiencies cause ↑ of phenylalanine in blood and ↑ phenyl compounds in urine*
      - ❖ *Branched chain ketoaciduria (maple syrup urine disease) — branched chain amino acids ↑ in blood and urine*
   b. Renal-normal plasma level but decreased renal threshold or reabsorption
      - ❖ *Cystinuria ↑ cystine, lysine, ornithine, arginine in urine*

METHODS

1. Screening Tests (*Initial Diagnosis*)
   a. Thin layer chromatography with ninhydrin
   b. Urine color tests
   c. Guthrie bacterial inhibition assays
   For PKU: positive when phenylalanine metabolites in blood overcome inhibition in agar and *B. subtillis* grows.
2. Quantitative Tests
   a. Ion-exchange chromatography
   b. High performance liquid chromatography (*HPLC*)
   c. Gas chromatography mass spectrometry (*GCMS*)

AMINO ACID LEVELS IN BLOOD

1. Homocysteine
   a. ↑ levels associated with ↑ risk of cardiovascular disease, stroke, Alzheimer's and osteoporosis
   b. ↑ levels if nutritional deficiencies of Vitamin B6, B12 and Folic Acid

Proteins

GENERAL

1. Most proteins are synthesized and catabolized in the liver

2. Basic unit - amino acids linked together by amide bonds to form protein

3. Protein breakdown in the body produces urea and ammonia; urea produced in liver and eliminated in urine by kidneys

METHODS : SERUM TOTAL PROTEIN

1. Kjeldahl
   a. Reference method
   b. Principle - measures nitrogen content
   c. Acid digestion converts nitrogen in protein to ammonium ion (NH_4^+) which is measured
   d. Difficult to perform, infrequently used

2. Biuret reaction
   a. Used most frequently
   b. Depends on presence of ≥2 peptide bonds which react to form a purple complex with copper salts in alkaline solution

METHODS : URINE AND CSF TOTAL PROTEIN

1. Dye — Coomassie brilliant blue

2. Turbidimetric methods
   a. Acid (*sulfosalicylic acid or trichloroacetic acid*) precipitates protein
   b. Measured spectrophotometrically or visually

METHODS : SPECIFIC SERUM PROTEINS

1. Dye-binding methods for albumin
   a. Bromcresol green (BCG)
   b. HABA (*2- [4' - Hydroxyazobenzene] - benzoic acid)*

2. Immunochemical methods for specific proteins other than albumin

PROTEIN ELECTROPHORESIS

1. Direction of migration of proteins in an electrical field determined by surface charge of protein
   a. Protein at pH higher than its isoelectric point is negatively charged and migrates toward anode (*positive charge*)
   b. Albumin (*smallest M.W.*) has largest number of free negative charges and migrates most rapidly traveling greatest distance from application point
   c. Urine protein electrophoresis same as serum except it must be concentrated before application

2. Electroendosmosis causes gamma globulins to migrate toward the cathode even though they are slightly negatively charged (*due to electrical charge on support medium)*

3. At pH 8.6, in order of migration, the 5 major bands are albumin, alpha-1, alpha-2, beta and gamma

4. Support media include cellulose acetate, agarose gel, and starch gel

5. Stains include Amido Black, Ponceau S and Coomassie Brilliant Blue

6. Relative concentration of each band determined by densitometry

7. Specimen collection and handling
   a. Plasma samples (*mistaken as serum)* result in fibrinogen peak migrating between gamma and beta fractions
   b. Recollect and repeat to verify that peaks not fibrinogen

CLINICAL SIGNIFICANCE

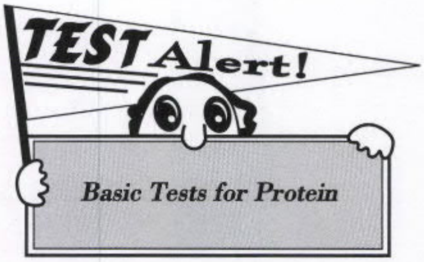
1. Plasma proteins - albumin and globulins
   a. Liver produces albumin, alpha-1, alpha-2 and beta globulins
   b. RE system produces gamma globulin (*antibodies secreted by plasma cells*)

2. Prealbumin
   a. Appears as a faint band on serum electrophoresis
   b. Used clinically to assess nutritional status

MAJOR SERUM PROTEIN ELECTROPHORESIS FRACTIONS

1. Albumin
   a. Largest plasma protein fraction (*52-62%)*
   b. Regulator of osmotic pressure
   c. Transport protein because of ease of binding with blood components
   d. Causes of ↓ values
      - ❖ ↓ *synthesis (liver impairment)*
      - ❖ ↓ *associated with edema, decreased osmotic pressure*
      - ❖ *Malabsorption or malnutrition*
      - ❖ *Nephrotic syndrome (renal loss)*
      - ❖ *Severe burns*
   e. ↑ values generally have no clinical significance *(hemoconcentration, dehydration)*

2. Alpha-1-globulins
   a. Alpha-1-antitrypsin (*AAT*)
      - ❖ ↑ *in acute phase and pregnancy*
      - ❖ ↓ *associated with emphysema in neonates*



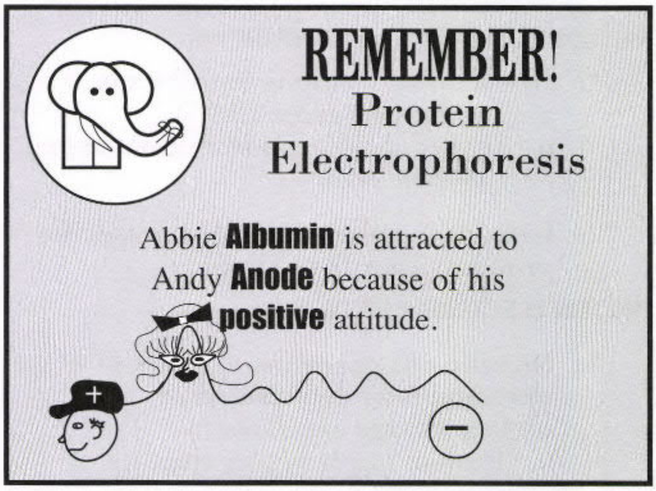
- b. Alpha fetoprotein (AFP)
  - ❖ ↑ *in amniotic fluid and maternal serum in neural tube defects (spina bifida)*
  - ❖ *liver cancer marker*
  - ❖ ↓ *in maternal serum during pregnancy associated with Down's syndrome*

3. Alpha-2-globulins
   - a. Haptoglobin
     - ❖ *Binds free hemoglobin*
     - ❖ ↑ *in acute phase and nephrotic syndrome*
     - ❖ ↓ *seen in transfusion reactions*
     - ❖ ↓ *in hemolysis and liver disease*
   - b. Ceruloplasmin
     - ❖ *Transports copper*
     - ❖ ↑ *in acute phase and pregnancy*
     - ❖ ↓ *Wilson's disease*

4. Beta globulin
   - a. Carrier proteins for iron (*transferrin*) and lipids (*lipoproteins*)
   - b. ↑ in:
     - ❖ *Elevated beta lipoprotein (LDL)*
     - ❖ *Iron deficiency anemia*

5. Gamma globulin
   - a. ↑ in:
     - ❖ *Chronic inflammation*
     - ❖ *Cirrhosis or viral hepatitis*
     - ❖ *Collagen diseases*
     - ❖ *Paraproteins (monoclonal bands, gammopathies)*
   - b. ↓ in congenital or acquired immuno-deficiency

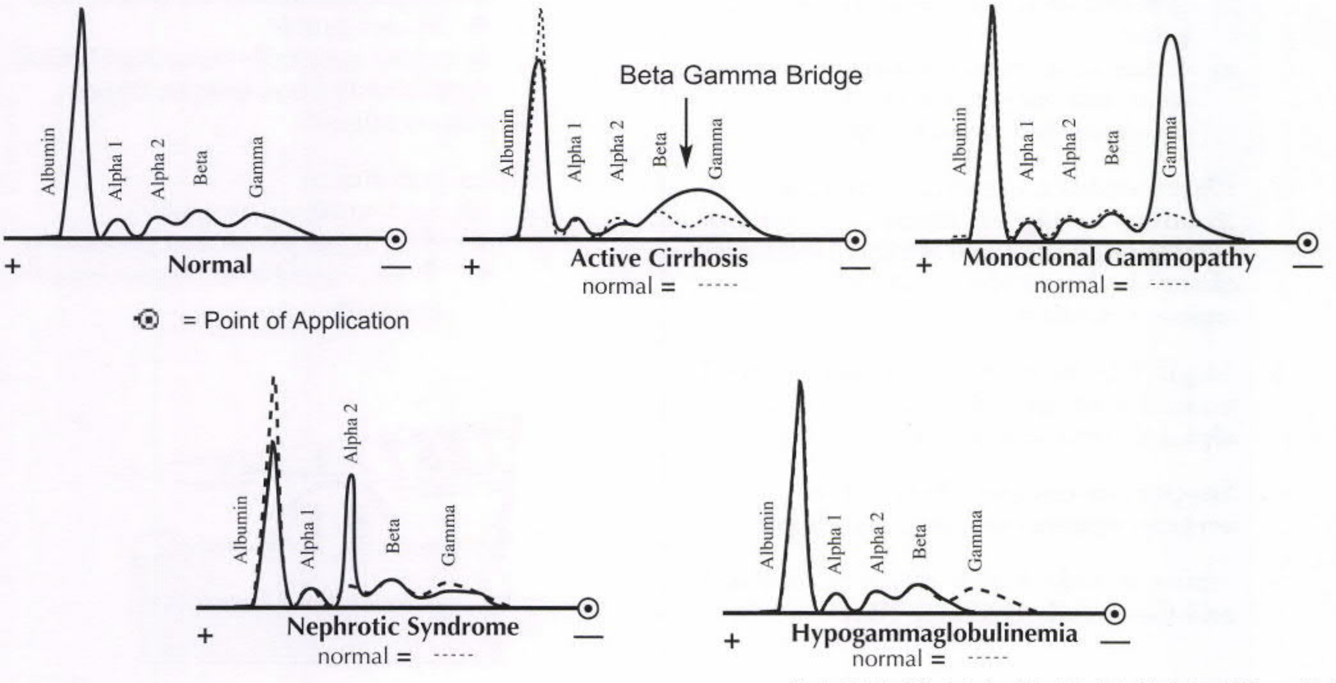
Associate Electrophoretic Pattern with Disease



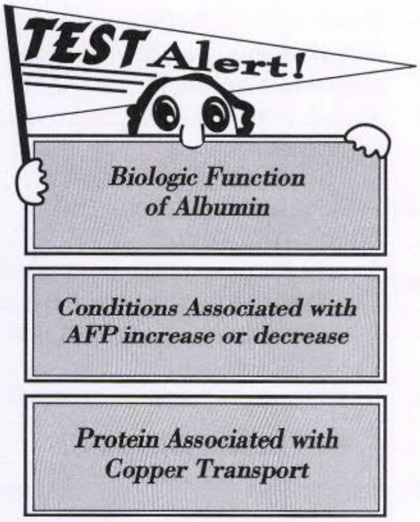
REMEMBER!
Protein Electrophoresis

Abbie **Albumin** is attracted to Andy **Anode** because of his **positive** attitude.

Some Common Protein Electrophoresis Patterns



• *Graphs Adapted with Permission from Helena Laboratories' Electrophoresis Reference Chart*



Biologic Function of Albumin

Conditions Associated with AFP increase or decrease

Protein Associated with Copper Transport

Enzymes

GENERAL

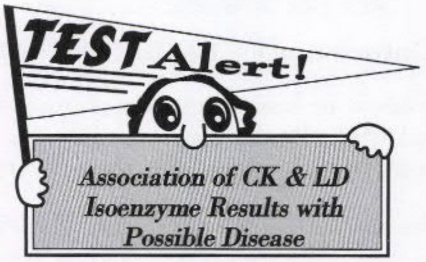
1. Organic catalysts responsible for most reactions in the body
2. Enzyme reactions in laboratory measurements affected by:
   a. Concentrations of reactants
   b. pH (*optimum pH varies with each enzyme)*
   c. Temperature
      ❖ *Optimum 37°C*
      ❖ *Rate doubles every ↑ 10 degrees*
   d. Ionic strength
   e. Cofactors and coenzymes
3. Enzyme inhibition by substances that can reduce the rate of the enzyme reaction
   a. Competitive inhibitor- binds free enzyme at the active site
   b. Noncompetitive inhibitor- binds at a site other than the active site
   c. Uncompetitive inhibitor- binds only to the enzyme substrate complex
4. Measurement
   a. Zero-order-kinetics — Large excess of substrate so that the amount of enzyme activity is only rate-limiting factor
   b. Catalytic activity rate (*not mass or concentration)* is directly measured
   c. One international unit (*U or IU*) = amount of enzyme that will cause utilization of substrate or production of product at the rate of 1 μM/ minute

Total CK (Creatine Kinase)

1. ↑ in muscle, cardiac or brain damage
2. Higher reference ranges in males due to greater muscle mass and physical activity

CK Isoenzymes

1. Different molecular forms of CK enzyme
   a. 2 subunits - M and B
      ❖ *CK1 = CK-BB - 2 B chains*
      ❖ *CK2 = CK-MB - 1 M & 1 B chain*
      ❖ *CK3 = CK-MM - 2 M chains*
   b. Cardiac muscle = CK-MM and CK-MB
   c. Skeletal muscle = CK-MM
   d. Brain, GI, colon, prostate, uterus = CK-BB
   e. Trauma to skeletal muscle causes ↑ in total CK and MB isoenzyme, but % activity MB is < 3% (*> 6% in MI*)



Association of CK & LD Isoenzyme Results with Possible Disease

LD (Lactate Dehydrogenase)

1. ↑ in:
   a. Myocardial infarction (MI)
   b. Liver disease
   c. Muscle trauma
   d. Renal infarct
   e. Hemolytic diseases
   f. Pernicious anemia
2. Sources of error
   a. Hemolyzed specimens
   b. Prolonged contact of serum to cells
3. Spectrophotometric method
   a. LD converts pyruvate to lactate while oxidizing NADH to NAD
   b. Rate of decrease in absorbance of NADH at 340 nm is proportional to LD activity
4. LD isoenzymes
   a. 2 chains *(M and H)*
   b. 4 subunits
   c. 5 forms *(tissue-specific)*

AST (Aspartate transaminase)

1. Found in cardiac muscle, liver, RBCs and other tissues
2. ↑ in MI, liver disease *(hepatocellular damage, cirrhosis, carcinoma)*, muscle trauma, renal infarct, hemolysis

ALT (Alanine transaminase)

1. ↑ in liver disease *(hepatocellular damage, cirrhosis, carcinoma)*
2. More liver-specific than AST

GGT (Gamma-glutamyl transferase)

1. ↑ in liver disease (*highest in biliary obstruction and cirrhosis*)
2. Often ↑ after alcohol intake
3. Spectrophotometric method — measure nitroaniline released when GGT acts on substrate gamma-glutamyl-p-nitroanilide

ALP (Alkaline Phosphatase)

1. Optimum pH = 10; Mg^{++} activation
2. Found in bone, intestinal mucosa, renal tubule cells, biliary tree (*liver*), leukocytes, placenta, some tumors
3. Isoenzyme separation: acrylamide gel, electrophoresis, chemical or heat (*56°C; 10 minutes):* Heat Stability:
   a. Regan *(cancer)* = rare, most heat stable
   b. Placental = most heat-stable of 4 most common
   c. Intestinal = inhibited by L-phenylalanine
   d. Liver = highest concentrations
   e. Bone = most heat-labile
4. ↑ in:
   a. Bone disorders with osteoblastic activity
      - ❖ *Paget's (highest ALP values)*
      - ❖ *Osteoblastic tumors*
      - ❖ *Rickets*
      - ❖ *Hyperparathyroidism*
   b. Growing children - rapid skeletal growth (*bone*)
   c. Disorders of hepatic biliary tree (*obstructive jaundice due to gallstones or malignancy*)
   d. Third trimester of pregnancy (*placenta*)
5. Methods
   a. Spectrophotometric
      - ❖ *ALP converts p-nitrophenyl phosphate to phosphate and P-nitrophenylate which is measured at 404-410 nm*
   b. Immunoassay for bone isoenzyme

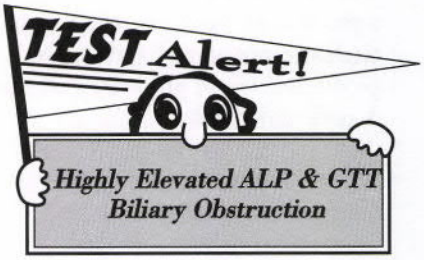
Correlate AST with Various Diseases

5'NT (5'-Nucleotidase)

1. ↑ in liver but NOT bone disease
2. ↑ ALP + Normal 5'NT = bone disease
3. ↑ ALP + ↑ 5'NT = liver disease

Amylase

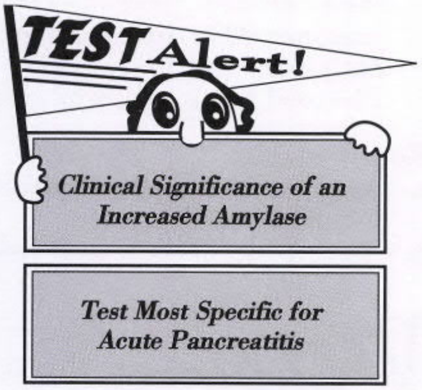
1. Produced in salivary and pancreatic glands
2. Requires Ca^{++} and Cl^- *(dilute elevated samples with saline not water)*
3. Only common enzyme normally excreted in urine
4. Highest elevations seen in pancreatitis and obstruction to pancreatic ducts (*malignancy*)
5. Lower elevations seen in obstruction of salivary glands (*mumps*)
6. Methods
   a. Amyloclastic — measure disappearance of starch substrate
   b. Saccharogenic — measure reducing sugars *(glucose and maltose)* produced by enzymatic action
   c. Chromolytic (*dye*) — measure absorbance of soluble dye split from insoluble amylase-dye substrate
7. Urinary amylase remains elevated longer than serum in pancreatitis
8. Opiates *(Ex. morphine)* cause elevation



Highly Elevated ALP & GTT Biliary Obstruction

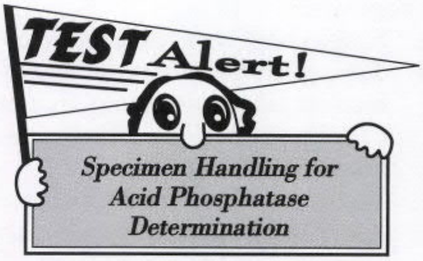
Lipase

1. ↑ in pancreatitis
2. Remains elevated longer than amylase
3. More specific for acute pancreatitis
4. Methods:
   a. Turbidimetric
   b. Older method: olive oil substrate; measure fatty acids product


TEST Alert!
Clinical Significance of an Increased Amylase
Test Most Specific for Acute Pancreatitis

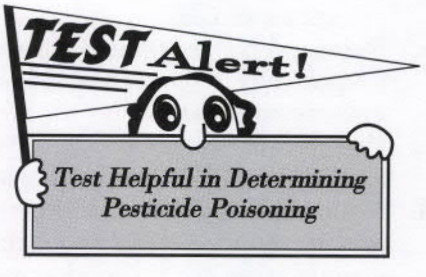
ACP (Acid Phosphatase)

1. Sources: primarily prostate; other tissues: erythrocytes, bone, liver, spleen, kidney, platelets
2. Clinical significance
   a. Highest elevations seen in metastasizing carcinoma of prostate; now use PSA instead
   b. ↑ in bone disease or cancers that metastasize to bone and in metastasizing breast cancer
   c. Tartrate-resistant portion elevated in hairy cell leukemia
   d. Presence in seminal fluid useful in forensic medicine for rape cases; now use PSA instead
3. Methods:
   a. Spectrophotometric for Total ACP
      - ❖ *Phosphate substrate (Ex. p-nitrophenyl phosphate) cleaved by ACP to give colored product (Ex. p-nitrophenol after OH^- added; yellow, read at 410nm)*
   b. Spectrophotometric for Prostatic ACP:
      - ❖ *Use substrates more specific for prostatic ACP (Ex. thymolphthalein monophosphate and alpha naphthyl phosphate)*
      - ❖ *Add tartrate buffer:*
        - ☞ Prostatic ACP inhibited by tartrate
        - ☞ RBC ACP not inhibited by tartrate
   c. Immunoassay for prostatic ACP
4. Specimen Collection and Handling
   a. Hemolysis results in falsely ↑ results
   b. Storage at room temperature results in loss of enzyme activity; must remove serum from cells ASAP and stabilize (*add disodium citrate monohydrate or ↓ pH to 5.4 with acetic acid)*


TEST Alert!
Specimen Handling for Acid Phosphatase Determination

Cholinesterase

1. Erythrocyte acetylcholinesterase and plasma pseudocholinesterase
2. Destroys acetylcholine after nerve impulse transmission
3. Severe ↓ results in serious neuromuscular effects; one of few enzymes in which ↓ is clinically significant
4. ↓ cholinesterase: organophosphate poisoning and genetic susceptibility to certain anesthetic agents


TEST Alert!
Test Helpful in Determining Pesticide Poisoning

Cardiac Markers to Evaluate Possible Acute Myocardial Infarction (AMI or MI)

1. Myoglobin
   a. Produced by muscles including heart
   b. ↑ in muscle damage including AMI
   c. ↑ in renal damage

   d. Rises within 30 minutes of AMI; peaks within 4-10 hours and returns to normal within 24 hours
   e. Absence rules out AMI but ↑ does not diagnose AMI because may be due to other muscle trauma

2. CK2 (*CK-MB*)
   a. Immunoassays: mass CK2 assays measure concentration rather than activity
   b. Rises within 6-10 hours of AMI; peaks within 24 hours; returns to normal in 2-3 days
   c. Replaced by Troponin for detection of AMI

3. Troponin
   a. Single best test for diagnosis of AMI
   b. Troponin (*Tn*) is complex of 3 muscle fiber proteins: troponin T (*TnT*), troponin I (*TnI*) and troponin C (*TnC*)
   c. Isoforms cTnT and cTnI are very specific to cardiac muscle and either may be used for detection of AMI
   d. cTnT and cTnI often called TnT and TnI or simply Tn
   e. Rises 4-8 hours after AMI; peaks at approximately 12-14 hours; remains elevated for up to 10 days
   f. Also used for cardiac risk stratification

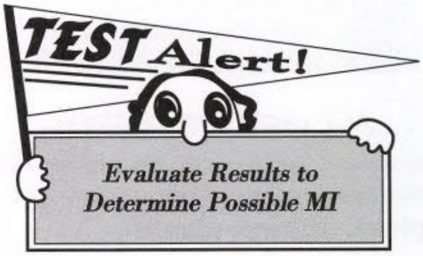
Cardiac Markers to Assess Congestive Heart Failure

1. B-type Natriuretic Peptide (*BNP*) and N-Terminal pro-BNP (*NT pro-BNP*)
   a. BNP and NT pro-BNP levels ↑ in congestive heart failure (*CHF*)
      ❖ *Levels correlate to classification of stages of CHF*
   b. Released by ventricular walls in response to hypertension and volume overload
   c. Pre-pro-BNP cleaved to BNP (*active*) and NT pro-BNP (*inactive*)
   d. Natriuretic because BNP ↑ Na^+ and water excretion and causes vasodilation to ↓ blood pressure
   e. BNP antagonist to renin-angiotensin-aldosterone system (*RAAS*) which ↑ blood pressure by vasoconstriction and retention of Na^+ and water
   f. NT pro-BNP cleared by kidneys so affected by renal function
   g. If BNP given as medication (*Natrecor®*) to ↓ blood pressure, must use NT pro-BNP to monitor ventricular BNP release
   h. Also used for risk stratification

Other Cardiac Risk Assessment Markers

1. hsCRP
   a. Sensitive marker for chronic inflammation
   b. Patient must be free of other inflammatory processes (*trauma, rheumatoid arthritis, infection, etc.*)
   c. Elevated levels potential risk factor

2. Homocysteine
   a. Amino acid associated with vitamin B6, B12 and folic acid
   b. Elevated levels potential risk factor



Evaluate Results to Determine Possible MI

Liver Markers

1. AST - highest values in hepatitis

2. ALT - highest values in hepatitis, liver specific

3. LD - found in many tissues other than liver *(ex., heart, skeletal muscle)*

4. ALP - biliary obstruction; may be slightly elevated in hepatitis

5. 5' NT - biliary obstruction

6. GGT - liver-specific; highest ↑ from biliary obstruction or after alcohol ingestion

REMEMBER!

Elevated Liver Enzymes are as Easy as **ABC**

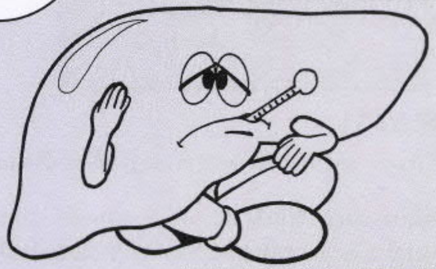
Alcoholism

Biliary Obstruction

Cirrhosis

REMEMBER!

Hepatitis vs. Obstruction



• Adapted with Permission from Sylvia Rayfield & Associates

HepATitis Herb
↑ AST
ALT

Plugged Up Paul
↑ ALP
5' NT =
5 Fingers

↘ ↑ GGT ↙

↑ Bilirubin

	AST	ALT	ALP	5'NT	GGT	BILI
Hepatitis	↑	↑			↑	↑
Biliary Obstruction			↑	↑	↑	↑

Muscle Disorders

1. Duchenne's Muscular Dystrophy, trauma, surgery, IM injections, trichinosis
2. ↑ in:
   a. CK
   b. AST
   c. Aldolase

Acute Pancreatitis

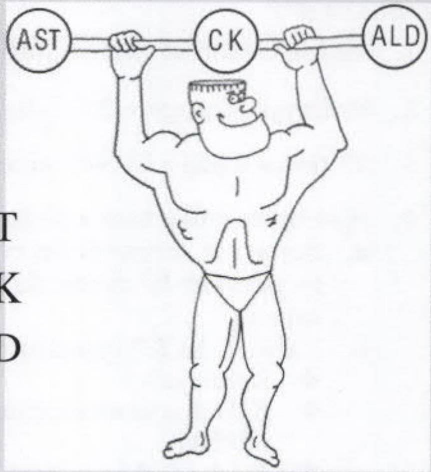
1. ↑ amylase (*serum and urine*); remains elevated longer in urine
2. ↑ lipase - more specific than amylase

I Am
Peter Pan
with Lips

↑ in Pancreatitis
Amylase
& Lipase

REMEMBER!

Muscle Man



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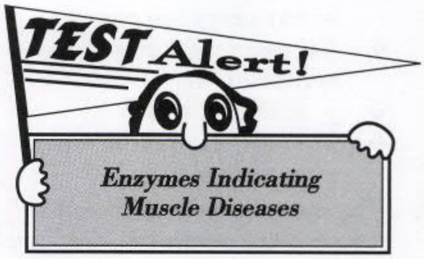


Electrolytes

SODIUM (Na^+)

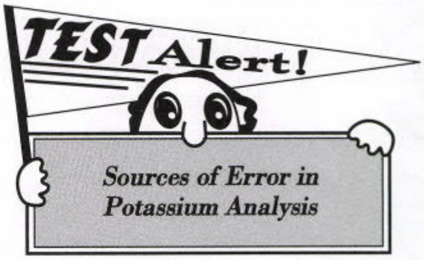
1. Major cation of extracellular fluid
2. 85% is reabsorbed in the kidney tubules
3. Reference range = 135-145 mM/L
4. Hyponatremia (↓ *serum* Na^+)
   a. Diabetic acidosis (metabolic)
   b. Diarrhea
   c. Addison's disease
   d. Renal tubular disease
5. Hypernatremia (↑ *serum* Na^+)
   a. Cushing's syndrome
   b. Dehydration
   c. Hyperaldosteronism (*causes ↑ renal reabsorption)*
   d. Insulin treatment of uncontrolled diabetes
6. Method: Ion-selective electrode
   a. Glass electrode selective for Na^+
   b. Direct reading
      ❖ *Sample not diluted*

   c. Indirect reading
      - *Sample diluted*
      - *Pseudohyponatremia if ↑ triglycerides or protein*

POTASSIUM (K^+)

1. Major cation of intracellular fluid
2. Reference range = 3.5-5.0 mM/L
3. 23 times higher in cells than in plasma
4. Specimen collection and handling
   a. Separate serum from cells quickly to prevent K^+ from shifting to serum
   b. False ↑ in K^+ *(pseudo-hyperkalemia)*
      - *Hemolysis*
      - *EDTA contamination (K^+ is in EDTA)*
      - *Prolonged tourniquet application*
      - *Excessive heel or finger squeezing in capillary specimens*
      - *Excessive fist clenching prior to venipuncture*
      - *$\geq$ 500,000 WBC*
      - *$\geq$ 700,000 platelet count*

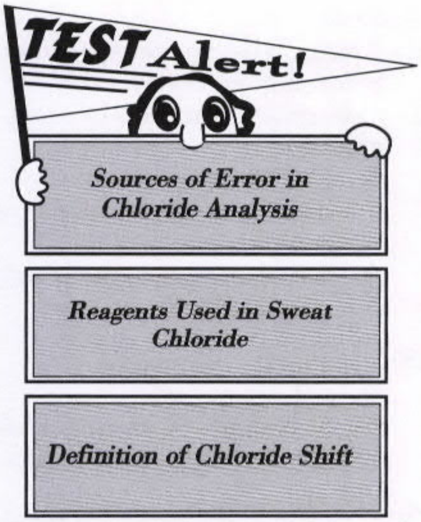
   c. Plasma and whole blood K^+, 0.1-0.7 lower than serum K^+ *(K^+ released from platelets during clotting)*
5. Hypokalemia (↓ *serum K^+*) and hyperkalemia (↑ *serum K^+*) may cause heart arrhythmias and/or neuromuscular symptoms including weakness and paralysis
6. Hypokalemia
   a. Insulin injections
   b. Alkalosis
   c. GI losses
      - *Diarrhea*
      - *Vomiting*
   d. Hyperaldosteronism (↓ *renal reabsorption)*
   e. Cushing's syndrome
7. Hyperkalemia
   a. Diabetic acidosis (*metabolic*)
   b. Intravascular hemolysis
   c. Severe burns
   d. Renal failure
   e. Addison's disease
8. Method: Ion-selective electrode
   a. Valinomycin membrane selectively binds K^+



Sources of Error in Potassium Analysis

CHLORIDE (Cl^-)

1. Major anion of extracellular fluid
2. Maintains hydration, osmotic pressure and the normal anion-cation balance
3. Reference range = 98-106 mM/L
4. Chloride generally follows Na^+ so ↑ and ↓ in same conditions
5. Hypochloremia (↓ *Cl^-*)
   a. Diabetic acidosis (*excessive acid production)*
   b. Chronic pyelonephritis
   c. Prolonged vomiting *(loss of gastric HCl)*
   d. Aldosterone deficiency
6. Hyperchloremia *(↑ Cl^-)*
   a. Prolonged diarrhea *(excess bicarbonate loss)*
   b. Renal tubular acidosis
   c. Adrenocortical hyperfunction
7. Methods:
   a. Ion-selective electrodes - solid-state electrodes containing AgCl (*silver chloride*) reference electrode
   b. Coulometric titration - generation of Ag^{++} ions which combine with Cl^- ions
   c. Colorimetry using $Hg(SCN)_2$ *(automated thiocyanate method)*; forms reddish color with peak at 480 nm
8. Sweat chloride
   a. ↑ in cystic fibrosis
   b. Sweat collected by iontophoresis using drug, pilocarpine, to induce sweating
   c. > 60 mM/L - cystic fibrosis
9. Chloride Shift
   a. Buffering system of the blood *(for acid-base balance)*
   b. HCO_3^- pulled out of erythrocytes and Cl^- moves into erythrocytes, resulting in ↓ serum Cl^-



Sources of Error in Chloride Analysis

Reagents Used in Sweat Chloride

Definition of Chloride Shift

CO_2 (TOTAL CARBON DIOXIDE)

1. $CO_2 + HCO_3^- + H_2CO_3$ = total CO_2
2. Reflects bicarbonate (HCO_3^-) concentration
3. Methods:
    a. Volumetric
    b. Manometric
    c. Colorimetric
    d. pCO_2 electrode measures change in internal pH due to CO_2

ANION GAP

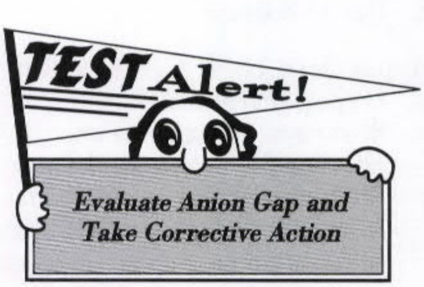
1. Calculation that reflects differences between unmeasured cations and anions; used as analytical QC for measuring all electrolytes
    a. If abnormal gaps for multiple patients, suspect problem with electrolyte measurements
2. Major unmeasured cations
    a. K^+
    b. Ca^{++}
    c. Mg^{++}
3. Major unmeasured anions
    a. Albumin
    b. Sulfate
    c. Phosphate
4. Two calculations *(with or without K^+)*:
    a. $[(Na^+) + (K^+)] - [(Cl^-) + (HCO_3^-)]$
    b. $(Na^+) - [(Cl^-) + (HCO_3^-)]$
5. Reference range
    a. 10-20 mM/L (*using equation 4a above*)
    b. 7-16 mM/L (*using equation 4b above*)
6. ↑ Anion gap
    a. ↑ in concentration of unmeasured anions
        - ❖ *Ethanol*
        - ❖ *Ketones*
        - ❖ *Lactic acid*
    b. ↓ in unmeasured cations
        - ❖ *Low serum Mg^{++}*
        - ❖ *Low serum Ca^{++}*
7. ↓ Anion gap
    a. ↓ in unmeasured anions- albumin loss
    b. ↑ in unmeasured cations
        - ❖ *High serum Mg^{++}*
        - ❖ *High serum Ca^{++}*
        - ❖ *Lithium therapy*
    c. Hemodilution

OSMOLALITY

1. Measure of total concentration (*number*) of dissolved particles in a solution (*molecular weight, size, density or type of particle does not matter*)
2. Can be measured directly - practical methods are freezing point depression (most precise) and vapor pressure depression — 2 colligative properties
3. One equation *(there are others that give similar results)*

$$\text{Calculated Osmolality} = 2\text{Na} + \frac{\text{Glucose}}{18} + \frac{\text{BUN}}{2.8}$$

4. Can compare calculated osmolality to measured osmolality; measured osmolality > 10 higher than calculated osmolality indicates presence of exogenous unmeasured anions (*methanol, ethanol, ketone bodies, etc.*)



Evaluate Anion Gap and Take Corrective Action

REMEMBER!

Conditions Causing ↑ in Unmeasured Anions
(ethanol, ketones, etc.)

S alicylate intoxication
L actic acidosis
U nmeasured ions
M ethanol
P olyethylene glycol
E thanol
D iabetic Ketoacidosis

MAGNESIUM (Mg^{++})

1. Ca^{++} channel blocking agent *(affects heart)*

2. ↑ in renal failure

3. ↓ in:
   a. Cardiac disorders
   b. Diabetes mellitus
   c. Diuretics, alcohol and other drugs

4. Methods
   a. Atomic absorption
   b. Colorimetric method - calmagite, formazen or methylthymol blue, or magon

CALCIUM (Ca^{++})

1. Combines with phosphate in bone

2. Controlled by 3 hormones:
   a. PTH (*parathyroid*) ↑ Ca^{++}
   b. Calcitonin inhibits bone reabsorption (↓ *Ca^{++}*)
   c. Vitamin D causes ↑ absorption in intestines (↑ *Ca^{++}*)

3. Hypercalcemia (↑ *Ca^{++}*) - muscle weakness, disorientation
   a. Hyperparathyroidism
   b. Cancer with bone metastasis
   c. Multiple myeloma
   d. Renal failure

4. Hypocalcemia (↓ *Ca^{++}*) - tetany
   a. Hypoparathyroidism
   b. ↓ serum albumin *(1 mg/dL Ca^{++} per 1 g/dL ↓ albumin)*
   c. ↓ vitamin D *(malabsorption, inadequate diet)* - impaired bone release, impaired renal reabsorption

5. Methods
   a. Atomic absorption spectroscopy - reference method
   b. Colorimetric method - most common
      ❖ *Ca^{++} reacts with o-cresolphthalein to form reddish complex*
      ❖ *8-hydroxyquinoline is added to remove Mg^{++}*

   c. ISE - measures ionized Ca^{++}
      ❖ *pH dependent*
      ❖ *Collection — anaerobically to prevent CO_2 loss (↑ pH)*

   d. Most methods measure total Ca^{++} including protein-bound Ca^{++} (↑ *protein causes* ↑ *Ca^{++}; ISE avoids problem)*

   e. Falsely ↓ if using EDTA (*purple top*) or oxalate; due to binding Ca^{++}

REMEMBER!

In Cases of Tetany, suspect Ca^{++} first, then Mg^{++} or K^{+}

PHOSPHOROUS

1. Majority of phosphate in body expressed as phosphorous; laboratory measures inorganic phosphorous (*PO_4*) only

2. Inverse relationship with Ca^{++} *(when Ca^{++} is ↑, PO_4 is ↓ and vice versa)*

3. ↑ PO_4
   a. Hypoparathyroidism
   b. Chronic renal failure
   c. Excess vitamin D

4. ↓ PO_4
   a. Hyperparathyroidism
   b. Impaired renal absorption

5. Methods
   a. Spectrophotometric methods use molybdate to combine with PO_4 ions
   b. Molybdenum blue is formed by the reduction of phosphomolybdate

REGULATORS OF CALCIUM AND PHOSPHORUS LEVELS

1. Vitamin D
   - a. Functions more like a prohormone than a vitamin
   - b. Exists in 2 forms:
     - *D2 (ergocalciferol) dietary form found in fish, plants, and fungus*
     - *D3 (cholecalciferol), most produced by photosynthesis in skin from exposure to sunlight but also found in dietary animal products*
   - c. Both forms metabolized to more active dihydroxy forms (1,25-(OH)$_2$D) in a 2-step process occurring first in liver (*producing 25 hydroxyvitamin D*) and then in the kidneys
   - d. 1,25-Dihydroxyvitamin D causes ↑ blood calcium and phosphorus by increasing intestinal Ca and PO$_4$ absorption and renal reabsorption and increasing mineralization during bone formation
   - e. Deficiency causes:
     - *Rickets: childhood disease characterized by softening and weakening of bones*
     - *Osteopenia and osteoporosis*
     - *Linked to other conditions such as hypertension, obesity, diabetes, cardiovascular disease, multiple sclerosis, cancer (colon and breast), autism, systemic lupus erythematosus (SLE), and other autoimmune diseases*
   - f. Appropriate test for assessing vitamin D stores:
     - *serum 25-hydroxyvitamin D (25-OH D2 & D3)*
   - g. Methods:
     - *Immunoassay*
     - *Liquid chromatography (high-performance liquid chromatography [HPLC] and liquid chromatography tandem mass spectrometry [LCMSMS])*

2. PTH (*Parathyroid Hormone)*
   - a. Synthesis by parathyroid glands stimulated by low Ca, suppressed by high Ca concentrations
   - b. Causes ↑blood calcium by
     - *Bone resorption*
     - *Renal tubular reabsorption of calcium*
     - *Stimulation of renal hydroxylation of 25-(OH)D to 1,25-(OH)$_2$D*
   - c. Intraoperative PTH monitoring:
     - *Rapid (POCT) assaying of PTH during surgery—use to determine if abnormal PTH producing tissue has been removed*
     - *Baseline plasma PTH and then at 5 and 10 minute intervals after removal of the parathyroid tissue*
     - *Look for >50% decline in PTH from 0 to 5 min postexcision*

3. Procalcitonin (PCT)
   - a. Normally made in thyroid and converted to calcitonin which causes ↓ blood Ca^{++}
   - b. In bacterial infection elevated levels (greater than 2.0 ng/mL) marker of high risk of sepsis

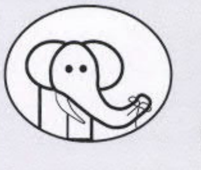
IRON

1. Over 65% of total body iron is in hemoglobin - O$_2$ transport

2. Transported by transferrin, haptoglobin and hemopexin

3. Stored as ferritin and hemosiderin

4. Methods
   - a. Serum iron - colorimetric - avoid hemolysis
   - b. TIBC (*total iron-binding capacity*)
     - *Reflects transferrin levels*
     - *Excess ferric salts are added to serum to saturate binding sites on transferrin*
     - *Unbound iron precipitated with magnesium carbonate*
     - *After centrifugation, supernatant analyzed for iron*
   - c. Direct methods for transferrin are immunochemical (*nephelometry*)
   - d. Ferritin
     - *Assess iron storage*
     - *Immunoassay methods*
     - *Sensitive for detection of iron deficiency*
     - ↑ *in infection, inflammation, chronic diseases*



REMEMBER!

Measure 25-OH vit. D

but 1,25-(OH)$_2$ vit. D most active form

Laboratory Assessment of Iron

DISEASE	SERUM IRON (*µg/dL*)	TRANSFERRIN SATURATION (%)	TIBC (*µg/dL*)	SERUM FERRITIN (*µg/dL*)
Normal	65-175 (M) 50-170 (F)	20-55	250-425	20-250 (M) 10-120 (F)
Storage Iron Depletion (No Anemia)	N	N	N	↓
Iron Deficiency Anemia	↓	↓	↑	↓
Anemia of Chronic Disease (Inflammation)	↓	↓	↓	↑
Thalassemia	↑	↑	↓	↑
Hemochromatosis	↑	↑	↓	↑
Sideroblastic Anemia	↑	↑	N	↑

Acid-Base Balance

HENDERSON-HASSELBALCH EQUATION

1. **Definition - logarithmic expression of ionization constant equation of a weak acid**
2. **Formula**
   - a. $pH = pKa + \log \frac{[HCO_3^-]}{[H_2CO_3]}$

     want this ratio to be $\frac{20}{1}$
   - b. pH proportional to:
     - ❖ $\log \frac{HCO_3^-}{pCO_2}$
     - ❖ $\frac{\text{kidney}}{\text{lungs}}$
     - ❖ $\frac{\text{metabolic}}{\text{respiratory}}$

SAMPLE COLLECTION AND HANDLING

1. **Anticoagulant - sodium heparinate (*heparin*)**
2. **Must use anaerobic collection for pH and blood gas studies**
3. **If blood is exposed to air (*bubbles in syringe; uncapped tube*):**
   - a. CO_2 and pCO_2 ↓
   - b. pH ↑
   - c. pO_2 ↑
4. **If testing prolonged *(> 15 minutes)* blood should be kept in cracked ice to prevent glycolysis, which leads to:**
   - a. CO_2 and pCO_2 ↑
   - b. pH ↓
   - c. pO_2 ↓

METABOLIC ACIDOSIS

1. Primary bicarbonate deficit *(↓ HCO_3^-)*
   - a. Diabetic ketoacidosis (↑ *acid production)*
   - b. Renal disease (↓ *H^+ excretion; decreased readsorption of HCO_3^-)*
   - c. Prolonged diarrhea *(excessive HCO_3^- loss)*
   - d. Late salicylate poisoning
2. Compensatory mechanisms
   - a. Primarily respiratory
     - ❖ *Hyperventilation*
     - ❖ ↓ *pCO2*
   - b. Some renal *(if kidney function normal)*
     - ❖ ↑ *excretion of H^+*
     - ❖ *Reabsorption of HCO_3^-*
3. Lab Findings
   - a. ↓ pH, HCO_3^-, CO_2 and pCO_2
   - b. Acid urine

METABOLIC ALKALOSIS

1. Primary HCO_3^- excess (↑ *HCO_3^-)*
2. Seen in:
   - a. $NaHCO_3$ infusion
   - b. Citrate *(anticoagulant in blood transfusions)*
   - c. Antacids *(contain HCO_3^-)*
   - d. Vomiting *(HCl loss; prolonged vomiting leads to alkalosis due to GI loss of H^+)*
   - e. K^+ depletion
   - f. Diuretic therapy
   - g. Cushing's Syndrome (↑ *mineralocorticosteroids)*
3. Compensatory mechanisms
   - a. Primarily respiratory
     - ❖ *Hypoventilation*
     - ❖ ↑ *retention of CO_2*

b. Some renal -
  - ❖ ↓ *excretion of H^+*
  - ❖ ↑ *excretion of HCO_3^-*

4. Lab Findings - ↑ pH, HCO_3^-, CO_2 and pCO_2

RESPIRATORY ACIDOSIS

1. Primary CO_2 excess *(↑ pCO_2)*
2. Seen in:
   a. Emphysema
   b. Pneumonia
   c. Rebreathing air (*paper bag*)
3. Compensatory mechanisms
   a. Mainly renal -
      - ❖ ↑ *H^+ excretion*
      - ❖ *HCO_3^- reabsorption*
   b. Some respiratory (*if defect is not in the respiratory center*)
4. Lab findings — ↓ pH and ↑ HCO_3^-, CO_2 and pCO_2

RESPIRATORY ALKALOSIS

1. Primary CO_2 deficit *(↓ pCO_2)*
2. Seen in:
   a. Hyperventilation (*blowing off too much CO_2*)
   b. Early salicylate poisoning
3. Compensatory mechanisms
   a. Mainly renal
      - ❖ ↓ *H^+ excretion*
4. Lab findings - ↑ pH and HCO_3^-,

   ↓ pCO_2 and CO_2

EVALUATING ACID-BASE DISORDERS

1. Look at pH; determine if acidosis or alkalosis
2. Compare pCO_2 and HCO_3^- to "normals"
   a. pCO_2 going opposite to pH = respiratory
   b. HCO_3^- going same direction as pH = metabolic
3. If pH normal, full compensation occurred
4. If main compensatory mechanism kicked in, but pH still out of normal range, partial compensation has occurred
5. Primary respiratory dysfunction results in change in pCO_2 (*seesaw*); main compensation is HCO_3^- (*metabolic*)
6. Primary metabolic dysfunction results in change in HCO_3^- (*swing*); main compensation is pCO_2 (*respiratory*)

BASE EXCESS / DEFICIT

1. Defined as amount (*dose*) of acid or alkali needed to return pH to normal
2. Calculated using pH and pCO_2
3. Assess metabolic component of acid-base disorder
   a. Positive value (*base excess*) = metabolic alkalosis
   b. Negative value (*base deficit*) = metabolic acidosis

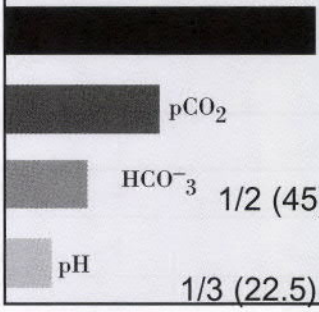
Blood Gas Reference Ranges

PARAMETER	DEFINITION	"NORMAL"
pH	Negative Log of H+	7.35-7.45
pCO_2	Partial Pressure or Tension of CO_2 in Blood	35-45 mm Hg
HCO_3	Bicarbonate - Calculated	22-26 mM/L
pO_2	Oxygen Tension - Partial Pressure of Oxygen	85-105 mm Hg

REMEMBER!

Blood Gas "Normals"

I like my oxygen at 100,



but 90 will do.

1/2 (90) = 45

1/2 (45) ≈ 23 (a little >1/2)

1/3 (22.5) ≈ 7.4 (a little >1/3)

Factors Affecting Blood Gas Analysis

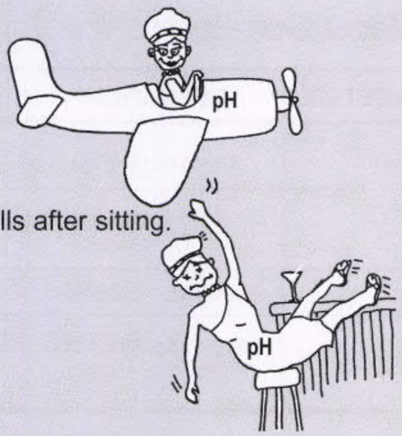
	pH	pCO_2	pO_2
Bubbles in Syringe	↑	↓	↑
Sample Sitting More Than 30 Minutes (Not on Ice)	↓	↑	↓

REMEMBER!

Factors Affecting Blood Gases

Let me introduce the characters who will help you remember blood gases:
Phonetia (pH),
Carbo (Bicarbonate - HCO_3^-), and Paco (pCO_2)

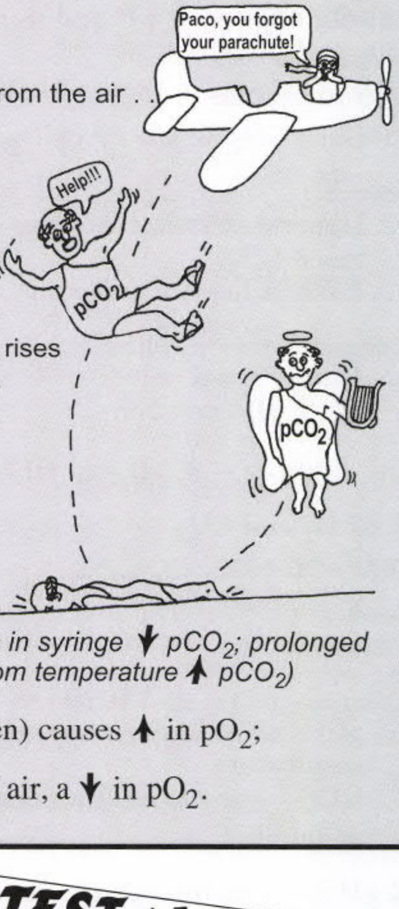
Phonetia flies through the air but . . .



. . . falls after sitting.

(Air bubbles in syringe ↑ pH, prolonged sitting at roomtemperature ↓ pH)

Paco falls from the air . .


Paco, you forgot your parachute!
Help!!!
pCO2
pCO2

. . . but rises after sitting.

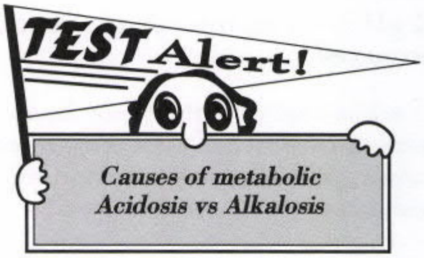
(Air bubbles in syringe ↓ pCO_2; prolonged sitting at room temperature ↑ pCO_2)

O_2 is simple: Exposure to air(oxygen) causes ↑ in pO_2;

prolonged sitting causes loss of air, a ↓ in pO_2.

Compensatory Mechanisms

Resp. Acidosis	Renal	↑ HCO_3
Resp. Alkalosis	Renal	↓ HCO_3
Metabolic Acidosis	Lung	↓ pCO_2
Metabolic Alkalosis	Lung	↑ pCO_2



Causes of metabolic Acidosis vs Alkalosis

REMEMBER!

Acid-Base Status

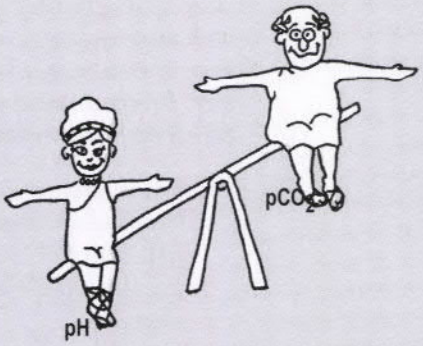
To determine acid base status (*respiratory or metabolic*) picture yourself in Rome. You are on a playground with Phonetia (*pH*), Carbo (HCO_3^-), and Paco (pCO_2).

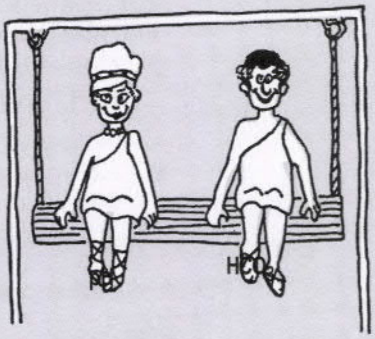
R ***Respiratory***

O ***Opposite***

M ***Metabolic***

E ***Equal***





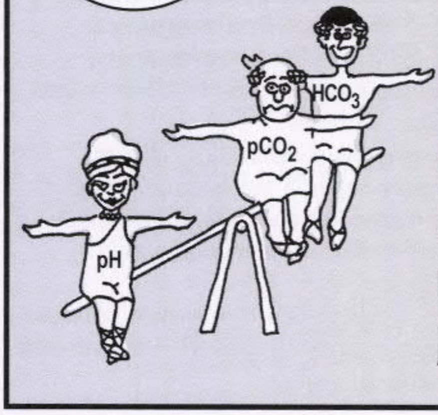
Phonetia and Paco hop on the seesaw and begin to play. Up and down, up and down. When the pH and pCO_2 are in opposite directions from "normal," the status is respiratory (*respiratory = opposite*).

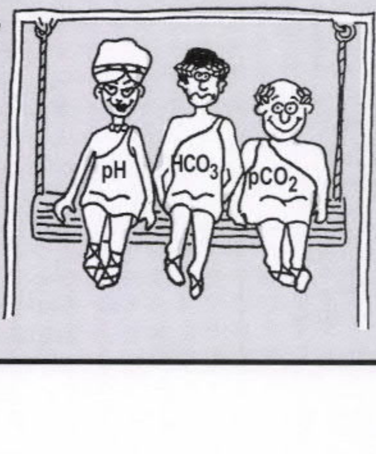
Phoentia tires of playing with Paco and runs off to join Carbo who is on a swing. Both go up and both go down, always together. When pH and HCO_3 are either both ↑ or both ↓, the status is metabolic (*metabolic = equal).*

pH > 7.45 = alkalosis
pH < 7.35 = acidosis

REMEMBER!

Compensatory Mechanisms





Compensation occurs in respiratory situations when Carbo gets mad at Phonetia for playing with Paco and hops on Paco's side of the seesaw! pH (*Phoentia*) goes up, pCO_2 and HCO_3^- (*Paco and Carbo*) go down. pH comes down, pCO_2 and HCO_3^- go up.

Compensation occurs in metabolic situations when Paco decides to crash the swinging twosome and hops on with Phonetia and Carbo. Now all go up or all go down.

Hey who needs Henderson or Hasselbalch!!!

ACID-BASE PROBLEMS

Determine the acid-base status in each of the following examples:

1.

$pH = 7.24$
$pCO_2 = 44$
$HCO_3^- = 18$

Answer: Metabolic Acidosis (*uncompensated*); Phonetia and Carbo are swinging down. pH < 7.35

2.

$pH = 7.52$
$pCO_2 = 44$
$HCO_3^- = 39$

Answer: Metabolic Alkalosis (*uncompensated*); Phonetia and Carbo are swinging up. pH > 7.45

3.

$pH = 7.26$
$pCO_2 = 56$
$HCO_3^- = 24$

Answer: Respiratory Acidosis (*uncompensated*); Phonetia and Paco are on the seesaw. pH < 7.35

4.

$pH = 7.52$
$pCO_2 = 28$
$HCO_3^- = 21$

Answer: Partially compensated respiratory alkalosis. Phonetia and Paco are seesawing. Carbo joins Paco to compensate.

5.

$pH = 7.39$
$pCO_2 = 25$
$HCO_3^- = 15$

Answer: Completely compensated metabolic acidosis or completely compensated respiratory alkalosis. For these situations, look at the pH. If it is on the low side of normal, choose acidosis. If it is on the high side of normal, choose alkalosis. In a like manner, completely compensated metabolic alkalosis cannot be distinguished from fully compensated respiratory acidosis. The Phonetia, Paco and Carbo story will work > 90% of the time in solving acid-base problems.

Hemoglobin Derivatives

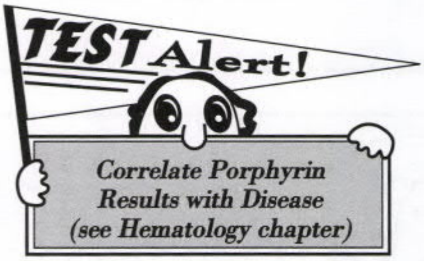
1. **Hemoglobin (*Hb*) breakdown products include porphyrins, bilirubin and urobilinogen**

LABORATORY ANALYSIS OF PORPHYRINS AND RELATED COMPOUNDS

1. **Urines with large amounts of porphyrins show a red or "port wine" color**
2. **Chromatography (*HPLC, ion-exchange*) to separate. All porphyrins have a characteristic pink fluorescence (*can be quantitated using a UV spectrophotometer or flurometer*)**
3. **Watson-Schwartz test**
   a. **Porphobilinogen (*PBG*) will react with Ehrlich's reagent, p-dimethyl-aminobenzaldehyde, to form red color**
   b. **Add chloroform to separate PBG from interfering compounds including urobilinogen *(UBG)***
      ❖ ***Color in chloroform top layer = UBG and other interfering compounds***
      ❖ ***Color in aqueous bottom layer = PBG***

LABORATORY ANALYSIS OF BILIRUBIN

1. **Diazotization methods**



Correlate Porphyrin Results with Disease (see Hematology chapter)

   a. **Classic method**
      ❖ ***Bilirubin + diazotized sulfanilic acid → azobilirubin (purple)***
      ❖ ***Total bilirubin (conjugated + unconjugated) reacts slowly with diazo reagent***
      ❖ ***Conjugated bilirubin (direct) reacts rapidly with diazo reagent in water* Jendrassik-Grof method uses *caffeine-benzoate* as accelerator**
   c. **Direct = conjugated = water soluble**
   d. **Indirect = unconjugated = relatively insoluble in water**

2. Direct Spectrophotometric method
    a. Newborns only; they lack interfering compounds

3. Specimen collection and handling
    a. Bilirubin is light sensitive, therefore sample should be stored in dark (*amber-colored*) glass
    b. Lipemia - falsely ↑ results
    c. Hemolysis - falsely ↓or↑ results depending on hemoglobin & bilirubin concentration

GENERAL INFORMATION

1. When unconjugated bilirubin is ↑, there will be a ↑ in urine urobilinogen *(due to ↑ amount reabsorbed from intestine and filtered by kidney)*

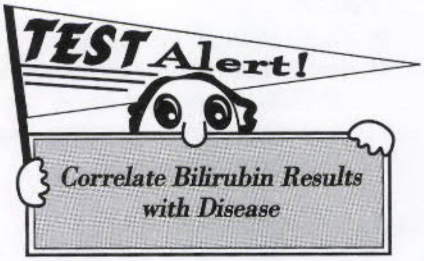
2. When conjugated bilirubin *(water soluble)* is ↑, it will appear in urine

CONDITIONS

1. Pre-hepatic jaundice *(i.e. hemolytic anemia)*
    a. ↑ red cell destruction → ↑ unconjugated bilirubin
    b. Liver function is normal; conjugation occurs at normal rate → normal conjugated bilirubin and no bilirubin in urine
    c. ↑ unconjugated bilirubin → ↑ urine urobilinogen

2. Hepatic jaundice *(i.e. viral hepatitis, cirrhosis)*
    a. ↑ unconjugated bilirubin, ↑ conjugated bilirubin and ↑ urobilinogen due to liver dysfunction
    b. ↑ conjugated bilirubin → ↑ urine bilirubin
    c. ↑ urine urobilinogen

3. Posthepatic (*obstructive*) jaundice
    a. Conjugated and unconjugated bilirubin cannot be metabolized properly; "back-up" into plasma
    b. ↓ urobilinogen (*due to blockage)* prevents conjugated bilirubin from entering intestine to be broken down into urobilinogen
    c. Stool may become clay colored


TEST Alert!
Correlate Bilirubin Results with Disease

MATERNAL AND NEWBORN TESTING DURING PREGNANCY

1. Maternal Serum prenatal testing at 16 weeks gestation
    a. Detection of neural tube defects (*such as spina bifida*)
        ↑ *alpha fetoprotein (AFP)*
    b. Detection of Down's Syndrome
        ❖ *Triple (HCG, AFP, unconjugated estriol) or Quad (add Inhibin A) screen*
            ↓ AFP
            ↓ estriol
            ↑ HCG
        Quad: (↑ Inhibin A)

Bilirubin and Disease States

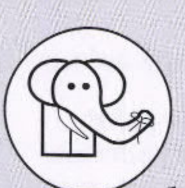
	Plasma/Serum		Urine	
DISEASE	**UNCONJUGATED BILIRUBIN**	**CONJUGATED BILIRUBIN**	**BILIRUBIN**	**UROBILINOGEN**
Prehepatic Jaundice (hemolytic anemia)	↑	N	0	↑
Hepatic (cirrhosis, viral hepatitis)	↑	↑	0 or ↑	↑
Posthepatic (obstructive jaundice)	N	↑	↑	↓

2. Premature labor or premature rupture of membranes (*PROM*)
   a. Premature Delivery – Fetal fibronectin (*ffN*)
      - ❖ *Vaginal swab specimen*
      - ❖ *Protein secreted at boundary of amniotic sac and uterus*
      - ❖ *Negative test indicates preterm delivery will not occur thus sparing preventative measures (MgSO4) with side effects*

   b. Premature rupture of membranes (*PROM*)
      - ❖ *Vaginal swab specimen*
      - ❖ *AmniSure™ detects amniotic fluid PAMG-1 present in cervico-vaginal secretions after rupture of fetal membranes*

3. Fetal newborn screening
   a. Dried blood spot specimen
   b. Tandem mass spectrometry used to screen for >25 genetic diseases (ex. PKU, congenital hypothyroidism, cystic fibrosis, sickle cell disease, other metabolic diseases)

REMEMBER!



Hemolytic Disease of the Newborn (*HDN*). . .

Since bilirubin cannot be conjugated in neonates:

- serum indirect (*unconjugated*) bilirubin ↑, conjugated bilirubin is normal for neonates
- unconjugated (*water insoluble*) cannot be excreted in the urine, so there is no urinary bilirubin
- unconjugated bilirubin cannot be broken down by intestinal bacteria so there is NO URINARY UROBILINOGEN (*appears as normal on "dipstick" - differs from other hemolytic processes where there is an ↑ in urinary urobilinogen*)

Toxicology

METHODS

1. Immunoassay

2. Chromatographic Techniques
   a. Thin-Layer Chromatography (*TLC*)
      - ❖ *Separates drugs for identification*
      - ❖ *Urine best specimen for detecting drugs*
      - ❖ *Limited sensitivity*
      - ❖ *Results should be confirmed with another method*

   b. Mass Spectrophotemtry (MS) as detector
      - ❖ After *separation & quantitation of drugs & metabolites by high performance liq. chrom. (HPLC or LC) or Gas Chromatog. (GC)*

   c. Gas Chromatography-Mass Spectrophotometry (*GC-MS*)
      - ❖ *"Gold-standard" technique for confirmation of screening methods*
      - ❖ *Highly sensitive and reliable*

ACUTE POISONING

1. Substances
   a. Cyanide
   b. Carbon monoxide- forms carboxyhemoglobin (*affinity for Hgb is 200 times the affinity for O_2*)
   c. Alcohols - Ethanol most common, enzymatic - alcohol dehydrogenase
   d. Heavy metals *(arsenic, mercury and lead)*
   e. Lead (***blocks heme pathway; ↑ ALA but not PBG***)
   f. Iron
   g. Salicylates - metabolic acidosis - respiratory alkalosis
   h. Organophosphates *(pesticides)*
      - ❖ *CNS symptoms*
      - ❖ ↓ *cholinesterase (RBC) and pseudocholinesterase (plasma)*
      - ❖ *see page 111*

   i. Acetaminophen - liver damage from accumulation of toxic metabolite 48 hours after ingestion; antidote (*N-acetylcysteine; Mucomyst™*) helpful if given in first 24 hours

Therapeutic Drug Monitoring (TDM)

1. Specimen collection
   a. Must wait until steady-state is reached to do therapeutic monitoring

   b. Takes $5^{1}/_{2}$ half-lives to reach steady state and it takes $5^{1}/_{2}$ half-lives to clear drug when medication stopped
   c. Trough specimen drawn immediately before next dose
   d. Most therapeutic ranges are for trough specimens
   e. Peaks for most drugs are drawn 1-2 hours after an oral dose, and vary for IV or IM procedures

2. Goal is to achieve therapeutic range, avoiding subtherapeutic or toxic concentrations

3. Each drug has own rate of absorption, peak time, extent of protein binding, metabolism and rate of excretion

4. Most common method: immunoassay

5. HPLC and GC
   a. Can measure parent drug and metabolites
   b. Disadvantages - expense, time and expertise required

6. Metabolism
   a. Most drugs metabolized in liver and excreted in urine
   b. Liver or kidney diseases affect drug levels
   c. If metabolites active, should monitor their levels also

ANTIBIOTICS

1. Aminoglycosides
   a. Generic names: Gentamicin, Tobramycin, Amikacin
   b. Inhibit bacterial protein synthesis; treat severe infections by gram-neg bacteria

2. Glycoprotein
   a. Generic name: Vancomycin
   b. Inhibit cell wall synthesis; treat severe infections by gram-pos bacteria

3. Monitor toxic range to prevent damage to hearing (*ototoxic*) and kidneys (*nephrotoxic*)

4. Measure peak and trough levels

ANTIARRHYTHMICS AND OTHER CARDIOACTIVE DRUGS

1. Digoxin
   a. If ↓ K^+ or ↓ Mg^{++}, may see toxic symptoms with therapeutic levels
   b. Treat overdose with Digibind *(antibody)*; cannot monitor digoxin levels after giving Digibind unless assay measures free digoxin only *(most assays do not)*

2. Quinidine
   a. Adding quinidine if patient taking digoxin causes ↑ digoxin

3. Procainamide
   a. Monitor metabolite NAPA also

4. Disopyramide

5. Lidocaine
   a. Very short half-life

ANTICONVULSANTS

1. Phenytoin *(Dilantin brand name)*
   a. Highly protein bound

2. Phenobarbital
   a. Induces enzymes to ↑ metabolism of all drugs

3. Valproic acid
   a. Adding valproic if patient taking phenobarbital causes ↑ phenobarbital

4. Primidone
   a. Monitor metabolite, phenobarbital, as well as parent drug primidone

5. Others: Carbamazepine and Ethosuximide

PSYCHOTROPICS (*ANTIDEPRESSANTS*)

1. Tricyclics
   a. Amtriptyline metabolized to nortriptyline *(can be given separately also)*
   b. Imipramine metabolized to desipramine *(can be given separately also)*
   c. Doxepin
   d. Methods
      - ❖ *Immunoassays measure total (parent plus metabolite)*
      - ❖ *Chromatography — LC, GLC (measure parent and metabolite separately)*

2. Lithium
    a. Used to treat bipolar disorders
    b. Methods
        - ❖ *ISEs*
        - ❖ *Atomic absorption*

BRONCHODILATORS

1. Theophylline
    a. Signs of toxicity include nausea, vomiting, headache, irritability, insomnia
    b. Severe toxicity can cause cardiac arrhythmias, seizures and death
    c. Caffeine is active metabolite in neonates *(monitor levels)*
    d. Methods
        - ❖ *Immunoassays (separate assays for theophylline and caffeine)*
        - ❖ *LC (monitor both theophylline and caffeine simultaneously)*

2. Caffeine
    a. Given to neonates

IMMUNOSUPPRESSANTS

1. Generic names
    a. Cyclosporine
    b. Tacrolimus
    c. Sirolimus
    d. Mycophenolic Acid (*MPA*)

2. Suppress rejection after organ transplants

3. Often used in combination

4. Whole blood specimen of choice except for MPA (*serum or plasma*)

5. May need multiple samples instead of trough collection—area under time/concentration curve reflects drug exposure

Renal Function

GENERAL INFORMATION

1. All non-protein nitrogens *(urea, creatinine, uric acid and ammonia)* are ↑ in plasma in renal impairment; referred to as azotemia

2. Best laboratory evaluation when renal impairment is suspected is glomerular filtration rate (*GFR*)

3. Creatinine clearance evaluates GFR *(more sensitive than BUN or creatinine)*

4. Creatinine clearance =

$$\frac{\text{U creat}}{\text{P creat}} \times \frac{\text{Volume 24 hr. Urine (mL)}}{\text{1440 (min/24 hr)}}$$

Creatinine clearance is expressed in mL/min

To correct for body surface area:

$$\text{Creat Clear} \times \frac{1.73}{\text{Area } (\textit{nomogram})}$$

5. eGFR *(estimated glomerular filtration rate)*
    a. More sensitive than creatinine clearance
    b. 2 different equations (*MDRD and CKD-EPI*) use serum creatinine, demographic info *(age, gender, race)*
    c. 24-hour urine collection not needed
    d. American Kidney Foundation recommendations to assess kidney damage
    e. Values above 60: report as > 60 ml/min

6. Urine Albumin
    a. Use with eGFR to stage and monitor chronic Kidney disease (*CKD*)

CREATININE

1. From creatine in muscle

2. Can also be measured to evaluate renal function; NOT as sensitive as GFR

3. Classic method is the Jaffe reaction
    a. Creatinine reacts with picric acid in alkaline solution to form a red-orange complex that absorbs light at 490-540 nm
    b. Interferents *(non-creatinine chromagens)* include glucose, acetoacetate and ascorbic acid

BLOOD UREA NITROGEN (*BUN*)

1. ↑ in impaired renal function
2. ↑ in high protein diet

3. Rises more rapidly than serum creatinine

4. Methods:
    a. Colorimetric method: urea reacts with diacetyl monoxime to form a colored complex
    b. Enzymatic method: Urease hydrolyzes urea into ammonia which is measured spectrophotometically or with an ISE
        - ❖ *Inhibited by the anticoagulant, sodium fluoride*

- ❖ *DO NOT use this anticoagulant for any enzyme analysis- may inhibit activity*

5. BUN/creatinine ratio is normally about 10:1-20:1

CYSTATIN C

1. Serum marker for GFR
2. Small protein produced by most nucleated cells in a consistent manner, unaffected by inflammation, gender, age, eating habits, or nutritional status
3. Method = immunoassay

URINE ALBUMIN

1. Units
   a. 24 hr collection: mg albumin/24 hrs
   b. Random sample: mg albumin/ gram creatinine
2. Albuminuria categories

Category	Urine albumin mg/24hr or mg/g creat.	Term
A1	< 30	N to mild ↑
A2	30-300	Moderate ↑
A3	> 300	Severely ↑

URIC ACID

1. End product of purine metabolism
2. ↑ in gout, renal failure, leukemia, and chemotherapy treatment
3. Colorimetric method
   a. Uric acid reduces phosphotungstic acid to tungsten blue measured spectrophotometrically
   b. Interferents include lipids and several drugs
4. Enzymatic assays are based on the uricase reaction in which allantoin and H_2O_2 are produced and H_2O_2 is coupled to give a colored product

AMMONIA

1. Derived from action of bacteria on contents of colon
2. Metabolized by liver normally
3. ↑ plasma ammonia toxic to the CNS
4. Hyperammonemia (↑ *ammonia*)
   a. Advanced liver disease *(most common cause)*
      - ❖ *Reye's syndrome*
      - ❖ *Cirrhosis*
      - ❖ *Viral hepatitis*
   b. Impaired renal function
      - ❖ *Blood urea is ↑ (↑ excretion into intestine, site of conversion to ammonia)*
5. Causes of false ↑ due to specimen collection and handling
   a. Failure to place sample on ice, centrifuge and analyze immediately *(nitrogenous constituents will metabolize to ammonia)*
   b. Poor venipuncture technique *(probing)*
   c. Incompletely filling collection tube

TEST Alert!
Specimen Collection for Ammonia Analysis

Endocrinology

GENERAL

1. Hypothalmus / Pituitary / End Organ System — Hypothalmus produces releasing hormone which stimulates pituitary to produce stimulating hormone that causes end organ to produce hormones or initiate a process *(see table page 128)*
2. Hyper and hypo conditions: end product hormone is ↑ (*hyper*) or ↓ (*hypo*)
   a. Primary caused by end organ problem
   b. Secondary caused by pituitary problem
   c. Tertiary caused by hypothalmic problem
3. Regulation — end organ product or process feeds back to hypothalmus and pituitary to stop production of releasing and stimulating hormones

THYROID HORMONES

1. Stimulate metabolic processes; necessary for normal growth and development
2. In the tissues T_4 is converted to T_3 (*physiologically active product*): T_4 concentration much higher than T_3

   a. **99.97% of T_4 bound to thyroxine-binding globulin (*TBG*), thyroxine-binding prealbumin (*TBPA*) and albumin; 0.03% is free**
   b. **T_3 is 99.5% bound and 0.5% free**

3. **Only free fractions metabolically active; bound is for storage and transport**

4. **Primary hyperthyroidism (↓ *TSH*; ↑ *T_4 and T_3*)**
   a. **Symptoms include weight loss, heat intolerance, hair loss, nervousness, tachycardia and tremor**
   b. **The most common cause is Grave's disease**
      - ***Autoimmune disorder***
      - ***Antibodies to thyroid-stimulating hormone (TSH) receptors***
      - ***Causes thyroid hyperactivity and suppression of TSH***
      - ***Lab findings***
         - ☞ **Normal or ↑ T_3 and ↑ T_4**
         - ☞ **↓ TSH**
   c. **Pregnancy**
      - ***TSH ↓ first trimester***
      - ***TBG ↑ due to estrogen***
      - ***FT4 and FT3 ↓ second and third trimesters***
      - ***Total T_4 and T_3 ↑***

5. **Primary hypothyroidism (↓ *T_4 and T_3*; ↑ *TSH*)**
   a. **Symptoms include fatigue, weight gain, decreased mental and physical output, cold intolerance**
   b. **Cretinism - congenital**
   c. **Myxedema - severe thyroid deficiency in adults**
   d. **Hashimoto's Thyroiditis**
      - ***Thyroid autoantibodies***
      - ***Lab findings***
         - ☞ **↓ T_3 and T_4**
         - ☞ **↑ TSH**

6. **Tests for Thyroid Function**
   a. **TSH**
      - ***Ultra-sensitive immunoassay***
      - ***Single best thyroid function test***
   b. **Total thyroxine (*T_4*)**
   c. **Free T_4**
   d. **(*Direct*) T_3 measures triiodothyrinine (*T_3*)**
   e. **T_3 uptake**
      - ***Indirect measurement of TBG***
      - ***No longer recommended***

ADRENAL CORTEX HORMONES

1. **Hypothalmus produces CRH that stimulates pituitary to produce ACTH that stimulates adrenal cortex to produce steroid hormones made from cholesterol**

2. **3 classes of steroids produced**
   a. **Mineralcorticoids**
      - ***Aldosterone***
   b. **Glucocorticoids**
      - ***Cortisol***
   c. **Sex hormones**
      - ***Androgens: testosterone***
      - ***Estrogens: estradiol***

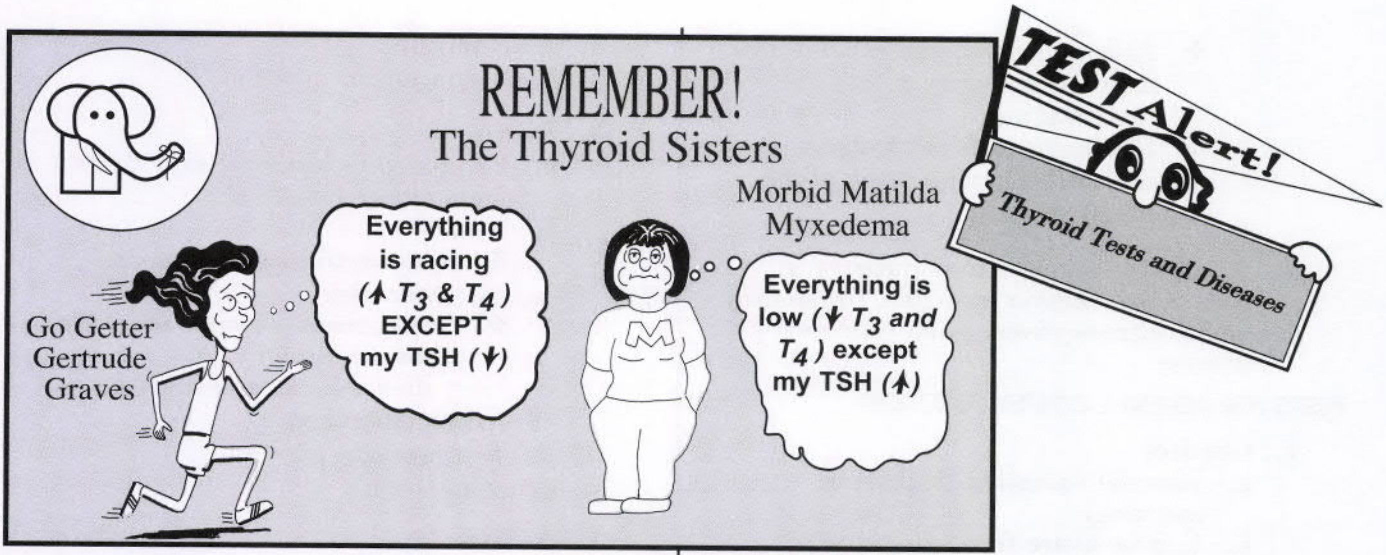
3. **Regulation — cortisol feedback to hypothalmus and pituitary to stop production of CRH and ACTH**

ALDOSTERONE

1. **Maintains blood pressure, promotes sodium reabsorption and potassium secretion in distal tubles and collecting ducts of the nephron**

HYPOTHALMUS / PITUITARY / END ORGAN SYSTEM

HYPOTHALMUS	PITUITARY	END ORGAN	PRODUCT / ACTION
TRH (thyrotropin releasing hormone)	**TSH** (thyroid stimulating hormone)	**Thyroid**	**T_4 and T_3**
CRH (corticotropin releasing hormone)	**ACTH** (adrenocorticotropic releasing hormone)	**Adrenal Cortex**	**cortisol, aldosterone, estrogens, testosterone**
GnRH (gonadotropin releasing hormone)	**LH** (leutinizing hormone) AND **FSH** (follicle stimulating hormone)	**Ovaries** OR **Testes**	**ovulation** OR **spermatogenesis**


REMEMBER!
The Thyroid Sisters
Go Getter Gertrude Graves
Everything is racing (↑ T_3 & T_4) EXCEPT my TSH (↓)
Morbid Matilda Myxedema
Everything is low (↓ T_3 and T_4) except my TSH (↑)
M
TEST Alert!
Thyroid Tests and Diseases

2. Regulation of secretion through renin-angiotensin system
   a. Renin converts angiotensinogen to angiotensin I which is rapidly converted to angiotensin II which stimulates cortex to produce aldosterone

3. Hyperaldosteronism (↑ *aldosterone*) - Conn's Disease
   a. ↑ Na^+
   b. ↓ K^+
   c. Hypertension

4. Hypoaldosteronism (↓ *aldosterone*) - Addison's disease
   a. ↓ Na^+ and Cl^-
   b. ↓ cortisol
   c. ↓ hemoglobin
   d. ↓ urinary steroids
   e. ↑ ACTH when primary hypoaldosteronism *(adrenal cortex problem)*
   f. ↓ ACTH if secondary *(pituitary)* or tertiary *(hypothalamus)* problems

CORTISOL

1. Functions
   a. Causes ↑ glucose through gluconeogenesis and decreased carbohydrate use
   b. Inhibits protein synthesis
   c. Immunosuppressive and anti-inflammatory

2. ↑ cortisol (*without diurnal variation)* - Cushing's syndrome
   a. Diabetes mellitus, ↓ plasma protein and hypertension
   b. Truncal obesity, facial hair, "buffalo hump", osteoporosis, scant menses

3. ↓ cortisol - Addison's disease (*see Aldosterone)*

ANDROGENS

1. Male sex hormones, secondary sexual characteristics

2. Secreted by testes, adrenals and ovaries

3. ↑ testosterone - precocious puberty in boys, testicular tumors; masculinization in females

4. ↓ in hypogonadism

5. 17-ketosteroids (*17-KS*)
   a. Metabolites of androgens
   b. Zimmermann reaction
      - ❖ *17-KS react with metadinitrobenzene in alcoholic alkali*
      - ❖ *Produces red-purple color*

ESTROGENS

1. Female sex hormones

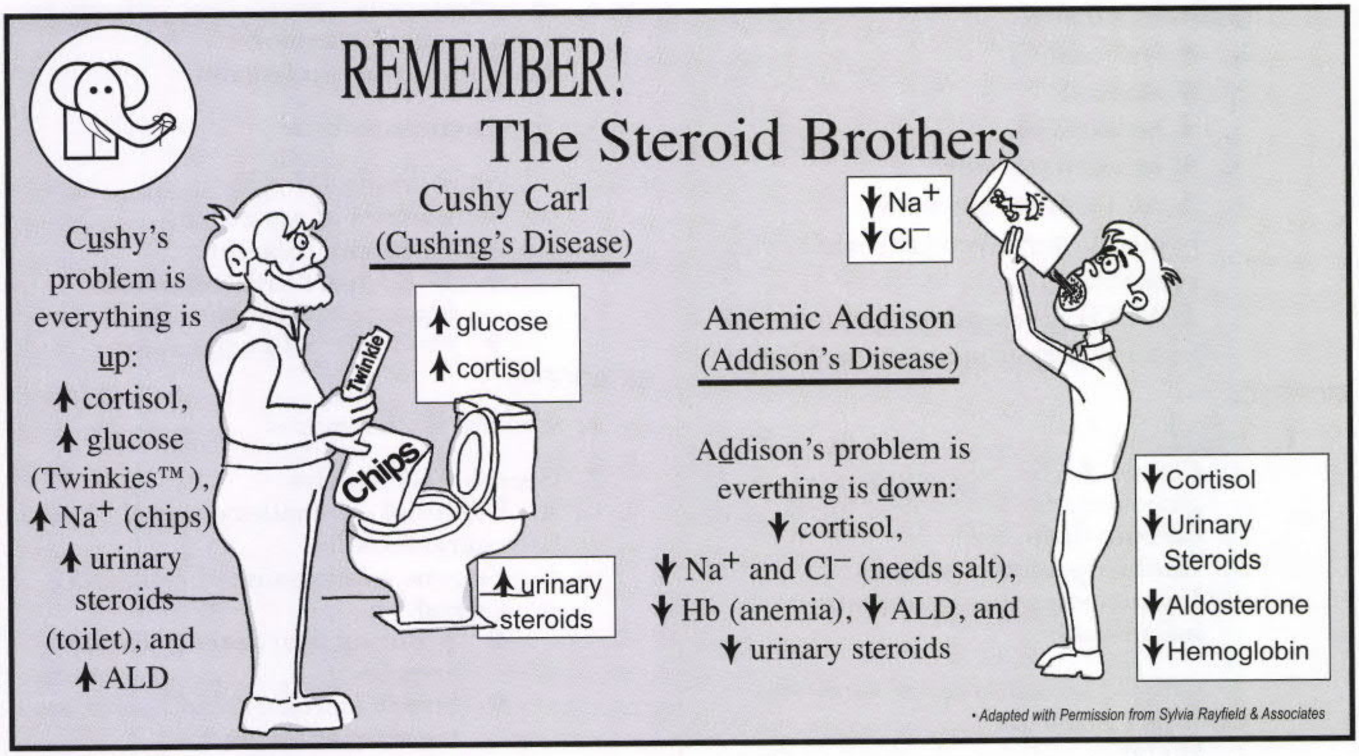
2. Secreted by ovaries
   a. Estradiol - secondary sexual characteristics
   b. Estrone - metabolite of estradiol
   c. Estriol
      - ❖ ↑ *during fetal development in pregnancy*
      - ❖ *Steady increase should occur in the third trimester*

- *24-hour urinary maternal estriol monitors integrity of feto-placental unit*
- *Decline or sudden change indicates a complication of the pregnancy*

3. ↑ estrogen - precocious puberty in girls, feminization in males, pregnancy, oral contraceptives, polycystic ovary disease

TESTS FOR ADRENAL CORTEX FUNCTION

1. Cortisol
   a. Diurnal variation (highest in morning)
   b. Can measure free *(unbound)* or total in serum, plasma or urine
2. Dexamethasone suppression
   a. Give dexamethasone to suppresses cortisol production
   b. Measure cortisol
   c. Interpretation
      - *Cortisol ↓ (suppressed) = normal*
      - *Cortisol ↑ (not suppressed) = Cushing's*
      - *Cortisol partially suppressed = depression, obesity, pregnancy, stress, infection*
3. Aldosterone
   a. ↑ in upright position
4. Renin
   a. Produced in kidneys; may draw from either renal vein
   b. Tests
      - *Renin activity (angiotensin I generation)*
      - *Direct renin (immunoassay for renin molecule)*
   c. Very unstable *(sample must be frozen immediately)*
   d. ↑ in upright position
   e. ↓ in Conn's
5. ACTH
   a. Distinguishes between primary and secondary hyperaldosteronism
6. Testosterone
   a. Hypothalmus / Pituitary / Testes and/or Adrenal Cortex
   b. Used for infertility testing
      - *Males: ↓ causes infertility*
      - *Females: ↑ causes infertility, hirsutism, masculinization*
7. Estrogens
   a. Measure estradiol and/or estriol in serum and urine


REMEMBER!
The Steroid Brothers
Cushy Carl
(Cushing's Disease)
Cushy's problem is everything is up: ↑ cortisol, ↑ glucose (Twinkies™), ↑ Na+ (chips) ↑ urinary steroids (toilet), and ↑ ALD
↑ glucose
↑ cortisol
Twinkie
Chips
↑ urinary steroids
↓ Na+
↓ Cl−
Anemic Addison
(Addison's Disease)
Addison's problem is everthing is down: ↓ cortisol, ↓ Na+ and Cl− (needs salt), ↓ Hb (anemia), ↓ ALD, and ↓ urinary steroids
↓ Cortisol
↓ Urinary Steroids
↓ Aldosterone
↓ Hemoglobin

• Adapted with Permission from Sylvia Rayfield & Associates

ADRENAL MEDULLA HORMONES : CATECHOLAMINES

1. Produced in chromaffin cells
    a. Epinephrine, norepinephrine and dopamine
    b. Homovanillic acid (*HVA*) = metabolite of dopamine
    c. Metanephrines and vanilylmandelic acid (*VMA*)= epinephrine metabolites
2. Pheochromocytoma
    a. Tumor of adrenal medulla
    b. Hypertension, headache
3. Neuroblastoma - fatal tumor in children
4. Tests for catecholamines
    a. Catecholamines *(plasma and/or urine)*
    a. Metanephrines in urine best screen for pheochromocytoma because catecholamine release is intermittent
    b. VMA - urine
    c. HVA - urine

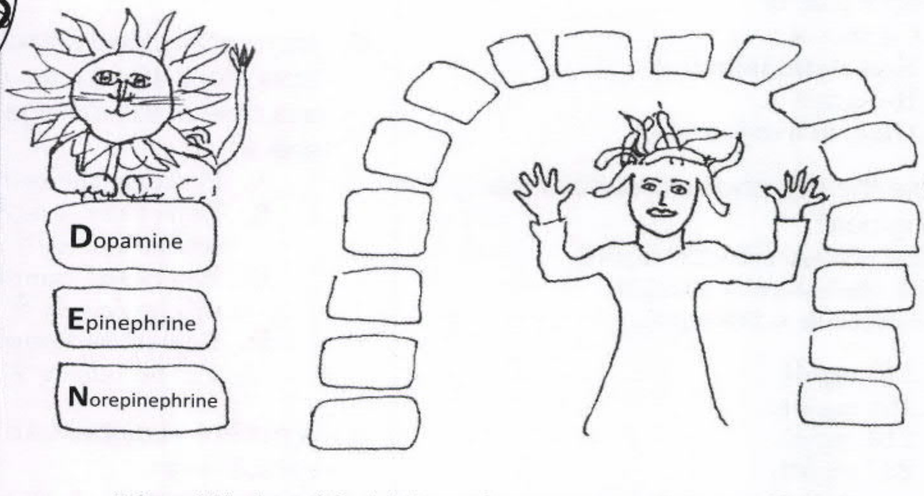
GASTROINTESTINAL HORMONES

1. Gastrin - ↑ in Zollinger-Ellison syndrome *(peptic ulcers, excessive acid secretion)*
2. Serotonin
    a. Vasoconstrictor found in platelets, brain and other tissue
    b. Increased production in tumors of chromaffin cells of GI tract
    c. Measure breakdown product, 5-hydroxy-indole-acetic acid (*5-HIAA*), in urine
    d. 5-HIAA falsely ↑ from some drugs or diet that includes bananas, pineapples and chocolate

TUMOR MARKERS

1. Most useful application of tumor markers is monitoring the course of treatment.
2. Common tumor markers and their related cancers (*see Immunology and Serology Chapter*)

REMEMBER!



Adrenal Medusa *(Medulla)* guards victims from the Lion's **D. E. N.** in the Catacombs *(Catecholamines)*

CHEMISTRY SAMPLE QUESTIONS

1. A patient is administered an oral glucose tolerance test with the following results:

   Fasting serum glucose- 128 mg/dl
   2 hour post-load serum glucose - 238 mg/d

   These results correlate with
   - A. Normal
   - B. Diabetes mellitus
   - C. Hypoglycemia
   - D. Malabsorption

2. A physician calls to ask assistance in choosing a test to monitor a diabetic patient's long term control. Which of the following would you suggest?
   - A. C-peptide of insulin
   - B. Glycosylated hemoglobin(s)
   - C. Fasting plasma glucose
   - D. Postprandial plasma glucose

3. Which of the following sets of test results indicates the greatest risk for coronary artery disease?

	Total Cholesterol mg/dL	HDL cholesterol mg/dL	LDL cholesterol mg/dL
A.	145	55	90
B.	165	60	105
C.	245	60	95
D.	345	30	205

4. Elevated conjugated billirubin in both serum and urine, decreased urine urobilinogen, and decreased fecal urobilin is characteristic of
   - A. Cirrhosis
   - B. Hemolytic jaundice
   - C. Hepatitis
   - D. Obstructive jaundice

5. Calculate the LDL cholesterol from the following data:

   Total cholesterol = 250 mg/dL
   HDL cholesterol = 40 mg/dL
   Triglyceride = 210 mg/dL

   - A. 140 mg/dL
   - B. 168 mg/dL
   - C. 210 mg/dL
   - D. 237 mg/dL

6. A 65 year old male visits his physician complaining of fatigue, shortness of breath and difficulty breathing. He is 6 feet tall and weighed 200 pounds for many years, but has recently gained 30 pounds. His ankles and hands are swollen and he complains of feeling bloated. His blood pressure is markedly elevated.

   What test should the physician order?
   - A. Troponin
   - B. Arterial blood gases
   - C. BNP or NT pro-BNP
   - D. Myoglobin

7. Which results will typically not be affected in a hemolyzed sample?
   - A. Acid phosphatase
   - B. Sodium
   - C. Iron
   - D. Potassium

8. Which enzyme is decreased in insecticide poisoning due to organophosphates?
   - A. Alkaline phosphatase
   - B. Amylase
   - C. Cholinesterase
   - D. Creatine kinase

9. The biuret method for determining serum total protein is dependent upon
   - A. Amino acid content
   - B. Number of peptide bonds
   - C. Nitrogen content
   - D. Protein precipitation

10. An arterial blood sample is received in the laboratory 45 minutes after collection with a bubble in the syringe. The technologist should
    - A. Perform the test immediately
    - B. Reject the sample because the pO_2 will be falsely ↓
    - C. Reject the sample because the pH will be falsely ↓
    - D. Reject the sample because the pCO_2 will be falsely ↓

11. A patient in diabetic ketoacidosis would exhibit a/an
    - A. $\uparrow pCO_2, \uparrow HCO_3^-, \uparrow pH$
    - B. $\downarrow pCO_2, \downarrow HCO_3^-, \uparrow pH$
    - C. Normal $pCO_2, \uparrow HCO_3^-, \downarrow pH$
    - D. Normal $pCO_2, \downarrow HCO_3^-, \downarrow pH$

12. The best laboratory test for detecting cystic fibrosis is
    - A. **Lipase**
    - B. **Sodium**
    - C. **Sweat chloride**
    - D. **Trypsin**

13. A 25 year old female visits her physician with the following symptoms: feeling tired all of the time, recently gaining 15 pounds, swollen neck, dry skin, hoarseness, and delayed reflexes. The physician ordered thyroid tests with the following results:

    TSH = 3.0 mIU/L (ref. 0.2-4.0 mIU/L)
    Free T_4 = 0.4 ng/dL (ref. 0.8-1.8 ng/dL)

    The physician performed a TRH stimulation test with the following results:
    30 minute TSH = 6.0 mIU/L
    60 minute TSH = 15.0 mIU/L

    The results indicate that the patient is suffering from:
    - A. **Primary hyperthyroidism**
    - B. **Primary hypothyroidism**
    - C. **Secondary or "pituitary" hypothyroidism**
    - D. **Tertiary or "hypothalamic" hypothyroidism**

14. A blood ammonia level was ordered on a patient with Reye's syndrome. The results on the clotted sample were normal. The most likely explanation for these results is
    - A. **Inappropriate sample collection**
    - B. **Incorrect diagnosis**
    - C. **Patient is under treatment**
    - D. **QC was out of acceptable limits**

15. A patient with ↑ serum levels of creatine kinase (CK), aldolase (ALD) and aspartate aminotransferase (AST) but normal levels of alanine aminotransferase (ALT) most likely has
    - A. **Hepatitis**
    - B. **Muscular dystrophy**
    - C. **Myocardial infarction**
    - D. **Pulmonary infarction**

16. In drug testing using thin layer chromatography, why is a volatile organic solvent mixed with urine specimens?
    - A. To preserve the drug
    - B. **To remove interferences**
    - C. **To extract the drugs**
    - D. **To enhance the effect of the drug**

17. Electrolyte values on 4 patients are as follows:
    1. **Na^+ 149 mmol/L; Cl^- 102 mmol/L; HCO_3^- 26 mmol/L**
    2. **Na^+ 153 mmol/L; Cl^- 105 mmol/L; HCO_3^- 28 mmol/L**
    3. **Na^+ 150 mmol/L; Cl^- 103 mmol/L; HCO_3^- 25 mmol/L**
    4. **Na^+ 151 mmol/L; Cl^- 104 mmol/L; HCO_3^- 27 mmol/L**

    Based on these results, what do you conclude about your electrolyte analyzer?
    - A. **There is a problem with the chloride analysis.**
    - B. **There is a problem with the bicarbonate analysis.**
    - C. **All analyses seem to be accurate.**
    - D. **There is a problem with the sodium analysis.**

18. Why should potassium levels be monitored prior to and during administration of IV insulin?
    - A. **Insulin causes potassium to move into cells which may cause a drop in potassium levels.**
    - B. **Insulin concentrates potassium in the serum causing hyperkalemia.**
    - C. **IV fluids dilute electrolyte values causing hyponatremia and hypokalemia.**
    - D. **Exogenous insulin causes hemolysis which falsely elevate potassium levels.**

ANSWERS AND RATIONALE

1. B

The American Diabetes Association (ADA) criteria for classification and diagnosis of diabetes is as follows:

One of the four criteria must be met to diagnose Diabetes mellitus
1. Diabetic Symptoms and random glucose ≥ 200 mg/dl
2. Fasting serum glucose ≥ 126 mg/dl
3. 2-hour postload serum glucose ≥ 200 mg/dl
4 A1c greater than or equal to 6.5%

In this case, two of the criteria are met. The oral glucose tolerance test is not necessary when the fasting glucose is ≥ 126 mg/dl.

2. B

Glycosylated hemoglobin is the specific hemoglobin fraction to which glucose molecules become irreversibly attached. Results of glycosylated hemoglobin are proportional to the average glucose level during the previous 1 to 3 month period. Option A is used to evaluate causes of fasting hypoglycemia. Options C and D give information about glucose levels for only a short period of time.

3. D

The treatment guidelines established by the Adult Treatment Panel of the National Cholesterol Education Program include recommendations that goals for cholesterol (*assuming no other risk factors are present*) should be less than 200 mg/dL for total cholesterol, <130 mg/dL for LDL cholesterol (*the "bad" cholesterol*), and >35 mg/dL for HDL cholesterol (*the "good" cholesterol).* All 3 values for Option D fall outside of the recommended levels and are considered independent risk factors. Options A and B have all values within the recommended levels. Although option C includes a total cholesterol of 245 mg/dL, an HDL cholesterol of 60 mg/dL and an LDL cholesterol of 95 mg/dL are not considered additional risk factors.

4. D

Obstruction in the bile duct results in conjugated bilirubin backing up into the circulation. Because it is water soluble, conjugated bilirubin is excreted into the urine. Since bile flow to the intestines is obstructed, urobilinogen and urobilin are found in the feces in less than normal amounts. Because of the liver damage, options A and C would result in increased levels of unconjugated and conjugated bilirubin in the serum. The increased conjugated fraction would be excreted in the urine. However fecal excretion of urobilinogen and urobilin would also be increased as the intestinal bacteria broke down the increased bilirubin. Option B would result in increased unconjugated bilirubin. Since the liver is undamaged, it could conjugate at the normal rate and there would be no excess to be excreted in the urine. There would be excess fecal urobilinogen due to the increased total bilirubin which would cause an increase in urine urobilinogen.

5. B

The Friedewald formula is:

$$\text{LDL chol} = \text{total chol} - \text{HDL chol} - \frac{\text{triglycerides}}{5}$$

$$= 250 - 40 - (210/5) = 168$$

6. C

The patient is exhibiting symptoms of congestive heart failure (*CHF*). BNP (*or NT pro-BNP*) levels correlate linearly to the New York Heart Association's classification of the stages of CHF. The most commonly used cutoff for the diagnosis is 100 pg/mL, but some studies have used lower levels. Myocytes in the ventricles produce a 134 amino acid peptide, pre-pro-BNP, which is cleaved to pro-BNP (*108 amino acids*) and a signal peptide (*26 amino acids*). Hypertension and volume overload cause increased tension and stretching of the ventricular walls, and in response pro-BNP is cleaved to BNP and N-terminal pro-BNP (*NT pro-BNP*) which are secreted into the blood. BNP is biologically active whereas NT pro-

BNP is inactive. BNP decreases blood pressure by vasodilation and renal excretion of sodium and water. Release of BNP is in direct proportion to ventricular wall tension, that is as the tension increases, the amount of BNP released also increases. Thus, BNP levels are an accurate reflection of the severity of heart failure.

7. B

Sodium is not affected in hemolyzed specimens. All other options are found in higher concentrations in the red cell than in plasma and are falsely increased in a hemolyzed sample. Hemolysis also falsely elevates magnesium and several enzymes such as LD, acid phosphatase, AST and ALT. The effect on bilirubin is dependent on the hemoglobin and bilirubin concentrations and may be method dependent.

8. C

Analysis for cholinesterase is unique in that a decreased rather than an increased result is significant. Under normal conditions this enzyme is synthesized in the liver and circulated in high levels. Cholinesterase is most often measured to diagnose and monitor insecticide poisoning and to detect inhibition by succinylcholine, a muscle- relaxant used in anesthesia. It is also decreased in liver disease and other chronic diseases.

9. B

The biuret method, which provides an accurate determination of total protein based on the number of peptide bonds which join amino acids together in protein molecules. Option A is not used. Option C is measured by the Kjeldahl method (*reference method*), but it is more difficult and time-consuming to perform. Option D is the principle of the turbidimetric methods for urine or spinal fluid protein analysis.

10. D

When blood is exposed to air (*air bubble in syringe*), the pCO_2 decreases and the pO_2 and pH increase.

11. D

Ketoacidosis is a metabolic disorder. In metabolic acidosis both pH and HCO_3 are decreased. In a compensated state pCO_2 would also be decreased.

12. C

Pilocarpine nitrate is electrically introduced into the sweat glands to induce sweating by a procedure called iontophoresis. Then sweat is assayed for chloride. In cystic fibrosis, both Na^+ and Cl^- are increased in sweat.

13. D

For the thyroid system, the hypothalmus produces TRH, which stimulates the pituitary to produce TSH, which stimulates the production of T_4 and T_3 by the thyroid gland. The decreased free T_4 level in this patient indicates hypothyroidism, but an elevated TSH is the expected result for primary hypothyroidism *(thyroid gland is dysfunctional)*. The TRH stimulation test involves injection of TRH and measuring baseline, 30 and 60 minute TSH levels. If the problem is a dysfunctional pituitary, the injected TRH will have no effect and the TSH levels will remain the same. If the problem is a dysfunctional hypothalmus *(tertiary hypothyroidism)*, the injected TRH stimulates the pituitary to produce TSH although this response is sometimes blunted or delayed. The results in this case are consistent with a diagnosis of tertiary or hypothalmic hypothyroidism.

14. A

Metabolism of nitrogenous constituents in blood is a source of plasma ammonia contamination. The anticoagulated blood sample (*usually collected in heparin*) must be put on ice immediately, centrifuged and analyzed without delay. Delays of greater than 15 minutes between sample collection and analysis have been shown to increase levels even when stored on ice. Option B is incorrect because in Reye's syndrome, ammonia blood levels are elevated since the liver is unable to convert it to urea. Options C and D could be true, but given that the collection process was incorrect, option A is a better response.

15. B

Option A is incorrect because the ALD would not increase and the ALT would be increased. Options C and D are incorrect because the ALD would not be increased.

16. C

When using thin layer chromatography to test for drugs in urine the drugs are extracted into an organic solvent after adjusting the pH with a buffer. Most drugs are more soluble in the organic compound than in the urine. The drug extract is concentrated by evaporation of the volatile organic solvent. The concentrated extract is then separated by TLC and the drugs identified.

17. D

Calculation of the anion gap may be used as a quality control monitor of electrolyte analysis. In this case all 4 samples show an increased anion gap (21, 20, 22, 20) with an elevated sodium. It is unlikely that all 4 patients would have conditions causing an increased sodium and/or increased anion gap. More likely is the probability that there is a problem with the analysis of sodium. Presumably the control values for sodium would also indicate a problem but there are occasions when the controls are within range but an instrumental problem exists and may be detected by calculation of the anion gap for all patient samples.

18. A

Insulin promotes the entry of potassium into liver and skeletal muscle cells. During administration of IV insulin potassium levels should be monitored to detect and treat hypokalemia if it develops.

MICROBIOLOGY / Bacteriology

by Mona Bakeer and Mary Lux

Bacterial Growth Requirements

1. Temperature
   a. Psychrophiles - cold loving; optimum temperature = 15°C
   b. Mesophiles - moderate temperature; optimum temperature = 37°C; (*most pathogenic organisms*)
   c. Thermophiles - heat loving; optimum temperature = 50-60°C

2. pH optimized for most
   a. Bacteria 6.5-7.5
   b. Fungi 5.0-6.0

3. Oxygen
   a. Aerobes - require O_2
   b. Facultative anaerobes - can grow with or without O_2
   c. Obligate anaerobes - harmed by O_2
   d. Formation of superoxide radicals, toxic; neutralized by catalase, peroxidase and superoxide dismutase (*possessed by aerobes and facultatives)*

4. Other atmospheric requirements
   a. Microaerophiles - prefer lower O_2 than in air
   b. Capnophiles - prefer higher CO_2 than in air
   c. Aerotolerant- do not require O_2 but not poisoned by O_2

CULTURE MEDIA

1. Must meet growth requirements

2. Agar - polysaccharide derived from marine algae
   a. Melts at 100°C
   b. Solidifies at approximately 45°C

3. Complex media - most common; made of peptones and extracts

4. Anaerobic media - contain reducing agents which bind with dissolved O_2 (*thioglycollate, cysteine*)
   a. Broth tubes should be heated prior to use to drive out O_2
   b. Gas pak envelopes
      - *Contain Na_2CO_3 and sodium borohydride*
      - *Add water - produces H_2 and CO_2 (aids in growth)*
      - *Palladium pellets catalyze the reaction*
      - *Some require hemin, Vitamin K, and yeast extract*

5. Typical incubation
   a. 5-10% CO_2 (*incubator or candle jar*)
   b. 35-37°C
   c. 50-70% humidity

6. May be selective and/or differential

STERILIZATION/INHIBITION TECHNIQUES

1. Heat - denatures protein
   a. Moist - autoclave (steam under pressure)
      - *15 lbs pressure/sq. in., 121°C, 15 minutes*
      - *QC - Bacillus stearothermophilus*
   b. Dry heat
      - *Flame, incinerator*
      - *Hot air oven 170°C, 2 hrs*
   c. Pasteurization, ultra high temperature
      - *140°C, 3 seconds*
      - *NOT sterilization*

2. Filtration
   a. Pore size 0.22 µ - 0.45 µ
   b. Used for sugar solutions, urea media, vaccines

3. Refrigeration - slows growth

4. Dessication - no multiplication, but organisms remain viable (*lyophilization*)

5. Osmotic pressure hypertonic solution
   a. Causes plasmolysis
   b. "Cured" meat, fruit preserves

6. Radiation
   a. Forms hydroxyl radicals
   b. Damages DNA

7. Disinfection
   a. Phenol - damages cytoplasmic membrane, denatures protein
   b. Halogens (*iodine and chlorine*) - oxidizers
   c. Alcohols - denature protein and dissolve lipids

STERILIZATION	DISINFECTION
Kills All Microorganisms (Including Spores and Viruses)	Inactivation or Inhibition of Microorganisms (May Not Affect Spores)
Examples: • Autoclave (121°C at 15psi for 15 min.) • Incineration • Filtration (Physically Removes Microorganisms)	Example: • Bleach (1:10 Hypochlorite)

TEST Alert!
Autoclave Pressure, Temperature and Time

Antibiotics and Their Actions

ANTIBIOTIC	EXAMPLES	ACTION	NOTES
ß-lactams	Penicillins Cephalosporins Carbapenams *(Imipenam)* Monobactams *(Azotreonam)* ß-lactamase Inhibiting Combinations *(Augmentin, etc.)*	Inhibits cell wall synthesis	Ceftriaxone, Cefotaxime
Glycopeptides	Vancomycin	Inhibits cell wall synthesis	Drug of choice for *Clostridium difficile* and MRSA
Aminoglycosides	Gentamicin Tobramycin Amikacin	Inhibits protein synthesis	Acts on 30S subunit; not active against anaerobes; used with a penicillin for *Enterococcus*
Tetracyclines	Tetracycline Doxycycline	Inhibits protein synthesis	Acts on 30S subunit; affects bone and teeth in children; may lead to superinfection of yeast
Chloramphenicol	Chloramphenicol	Inhibits protein synthesis	Acts on 50S subunit; can cause aplastic anemia
Macrolides	Erythromycin Clindamycin	Inhibits protein synthesis	Acts on 50S subunit; clindamycin for GP and GN anaerobes
Quinolines	Ciprofloxacin Norfloxacin	Inhibits nucleic acid synthesis	For *Pseudomonas aeruginosa* and other aerobes
Sulfa Drugs *(Sulfonomides)*	Sulfamethoxazole	Analogue of PABA *(intermediate in folic acid synthesis)*	For UTI, enteric infections; used with trimethoprim *(Bactrim, etc.)*
Streptogramins	Quinupristin/dalfopristin	*Inhibits protein sythesis*	*For GP, especially vancomycin resistant Enterococcus faecium*
Oxazdidinones	Linezolid	*Inhibits protein sythesis*	*For GP, including those resistant to other antibiotics*

Antibiotics/Susceptibility Testing

ANTIMICROBIAL THERAPY

1. **Narrow spectrum - only certain groups covered**
2. **Broad spectrum - Gram pos (*GP*) and Gram neg (*GN*) coverage**
3. **Selective toxicity - action against microbe only without injuring cells of host**
4. **Bactericidal action - kills bacteria without host immune help**
5. **Bacteriostatic action - reversible inhibition (*ultimate destruction depends on host defenses*)**
6. **Drug combination**
    a. **Synergism - combined better than the sum: 1 + 2 = 4**
    b. **Antagonism - one decreases activity of other: 1 + 2 = 1**

SUSCEPTIBILITY TESTING

1. Kirby-Bauer Method
   a. Disk diffusion
   b. Mueller-Hinton agar (*MH*)
   c. Depth = 4mm
   d. pH = 7.2-7.4
   e. Physiologic concentration of Ca^{++} and Mg^{++}
   f. 35°C, ambient air
   g. 10^8 organisms (*McFarland 0.5*)
   h. QC weekly and with each new lot of agar or discs (*E. coli, S. aureus, P. aeruginosa)*

2. Broth methods
   a. MIC (*minimum inhibitory concentration)*
      - *Lowest concentration of drug that prevents in vitro growth*
      - *First dilution tube with no visible growth*

   b. MBC (*minimum bacteriostatic concentration)*
      - *Lowest concentration that results in >99.9% killing*
      - *Subculture tubes near MIC to find first plate with no growth*

3. E-test
   a. MIC on a stick
   b. Plastic strips impregnated with antimicrobials

4. Modified methods for testing slow-growing or fastidious bacteria
   a. Haemophilus test medium
   b. Supplemented MH for *S. pneumoniae*
   c. Supplemented GC agar base for *N. gonorrhoeae*

5. Extended spectrum beta-lactamase (*ESBL*)
   a. Enzymes for resistance to extended-spectrum (third-generation) of cephalosporins and monobactams but do not affect cephamycins
   b. Enzyme activity may vary
   c. If an ESBL is detected, all penicillins, cephalosporins, and aztreonam should be reported as resistant
   d. Especially consider *Escherichia* and *Klebsiella* as potential ESBL-producing organisms

6. D-test
   a. Used for detection of inducible clindamycin resistance.
   b. Clindamycin 2µg disks and erythromycin 15 µg disks used
   c. Inducible strains form a "D"-shaped zone of inhibition

7. Detection of MRSA
   a. Zone of ≤10 mm with an oxacillin (1 µg) disk on Mueller-Hinton
   b. Molecular tests for mecA gene

8. Vancomycin-Resistant Enterococci (VRE)

9. Carbapenem resistant enterobacteriaceae (CRE)
   a. Enzymes resistant to Carbapenem antibiotics
   b. *Klebsiella* spp. especially *K. pneumonia (KPC). E. coli, Enterobacter*
   c. Resistant to penicillin, cephalosporins, carbapenem and aztreonam
   d. Modified Hodge test and chromogenic media

10. Automated AST
   a. BD Phoenix
   b. Microscan Walkaway
   c. TREK sensititre
   d. Vitek 1 and 2

Sources of Error: Disk Diffusion

ABNORMAL RESULT	PROBABLE CAUSE
Tetracycline Zone Too Large and Clindamycin Too Small with *E. coli* or *S. aureus* Controls	pH of agar too low
Tetracycline Zone Too Small and Clindamycin Too Large with *E. coli* or *S. aureus* Controls	pH of agar too high
Aminoglycoside Zone Too Small with *P. aeruginosa* Control	Ca^{++} and/or Mg^{++} too high in agar
Aminoglycoside Zone Too Large with *P. aeruginosa* Control	Ca^{++} and/or Mg^{++} too low in agar
Zones Universally Too Large on Control Plates	Inoculum too light Nutritionally poor medium Slow-growing organism (not seen with controls) Agar depth too thin
Zones Universally Too Small on Control Plates	Inoculum too heavy Agar depth too thick
Methicillin Zone Decreasing over Days or Weeks with Control Organisms	Methicillin degrading during refrigerator storage
Methicillin Zone Indeterminate in Disk Test	Methicillin being degraded by strong ß-lactamase producing Staphylococci
Colonies within Zone of Inhibition	Mixed culture Resistant mutants within zone
"Zone within a Zone" Phenomenon	A swarming *Proteus* Feather edges of zones around penicillin or ampicillin disks usually occur with ß-lactmase neg. strains of *S. aureus* ß-lactamase pos. *H. influenzae* with penicillin or ampicillin

Media

Routine Media

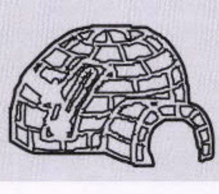
MEDIA	PURPOSE
Blood Agar (BA, BAP)	Most Bacteria; Determines Hemolytic Reactions
Chocolate Agar	*Haemophilus* and *Neisseria sp.*; Enriched with Hemoglobin or IsoVitalleX
Phenylethyl Alcohol Agar (PEA)	Selects for Gram Positive Cocci and Anaerobic Gram Negative Bacilli
Columbia Colistin-Nalidixic Acid (CNA)	Selects for Gram Positive Cocci
Thayer-Martin Agar	*N. gonorrhoeae* and *N. meningitidis*
CAMPY-Blood Agar	*Campylobacter sp.*
Thioglycolate Broth	"Back-Up" for Anaerobes
Lowenstein-Jensen Agar	*Mycobacterium sp.*
Middlebrook 7H10 Agar	*Mycobacterium sp.*
Petragnani Agar	*Mycobacterium sp.*

Anaerobic Media

MEDIA	PURPOSE
Bacteroides Bile Esculin Agar (BBE)	Selects for *B. fragilis* Group (Black Colonies)
Kanamycin-Vancomycin Laked Blood (KVLB)	*Bacteroides sp.* (Enhances Pigment Production)
Cycloserine-Cefoxitin Fructose Agar (CCFA)	*C. difficile*
CDC Anaerobic Blood Agar	Anaerobes (Enriched with Hemin, Cystine, and Vitamin K)
Cooked or Chopped Meat Medium	Anaerobes

COLD ENRICHMENT

- *Listeria monocytogenes*
- *Yersinia*

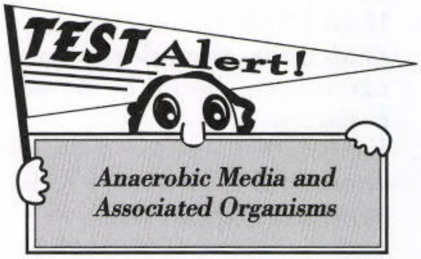


Special Media

MEDIA	PURPOSE
Bordet-Gengou Agar	*B. pertussis*
Buffered Charcoal Yeast Extract (BCYE)	*Legionella sp.*
Cystine-Glucose Agar	*F. tularensis*
Fletcher's Medium	*Leptospira*
Skirrow Agar	*Helicobacter pylori*
Thiosulfate Citrate-Bile Salts Sucrose (TCBS)	*Vibrio sp.*
Vaginalis Agar (V-Agar) (human blood)	*Gardnerella vaginalis*
Cystine-Tellurite Blood (Tinsdale)	*C. diphtheriae* (Black Colonies)
Loeffler's Medium	*C. diphtheriae* (Enhances Grouping and Metachromatic Granules)

Select Biochemical Reactions

Biochemical Test Medium	Uninoculated Color or Negative Reaction	Positive Reaction
Carbohydrate fermenatation with phenol red	Red	Yellow
Esculin	Nondescript	Black
Hippurate hydrolysis	No change	Purple
Motility	Growth along stab line	Blurred stab line
6.5% NaCl Broth	Clear	Turbid
Phenylalanine or trytophan deaminase	Nondescript	Green surface after 10% FeCl3
Urea	Nondescript	Bright pink



Anaerobic Media and Associated Organisms

Special Media and Associated Organisms Especially Legionella

Specimen Collection and Handling

GENERAL

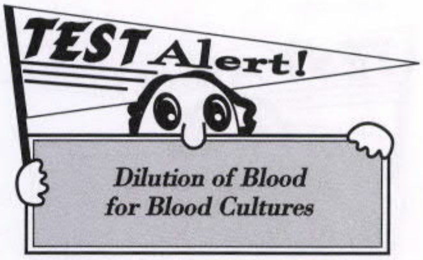
1. **Material from infection site**
2. **Optimal time** (***ex.: Salmonella typhi- culture blood first week, culture stool second and third weeks***)
3. **Appropriate collection devices**
4. **NEVER refrigerate spinal fluids, anaerobic or GC specimens**
5. **Collect prior to antibiotic therapy**
6. **Set up within 2 hours of collection**

CRITERIA FOR REJECTION

1. **Preservatives used**
2. **Insufficient quantity**
3. **Dry swab**
4. **Leaky containers - contaminated specimen as well as biohazard**

BLOOD CULTURE COLLECTION

1. **Must prep skin properly with alcohol (*70%*) and iodine**
2. **Best time to draw is just prior to fever spike**
3. **Draw at least 2 cultures, but no more than 3 in a 24 hr period**
4. **May use antibiotic removal device (*ARD*) or resin bottles if patient on antibiotics**
5. **Isolator® best for fungi and acid fast organisms**
6. **Need 1:10 dilution of blood to broth; on adults draw at least 10 ml (5ml for pediatric bottles)**


TEST Alert!
Dilution of Blood for Blood Cultures

TEST Alert!
Collection and Handling of Blood Culture Specimens

Diagnostic Methods

MICROSCOPY

1. **Light microscopy**
    a. **Resolving power - 0.2 µm**
    b. **Ocular lens = 10X; oil immersion lens = 100X**
2. **Darkfield - for spirochetes; reflected light**
3. **Fluorescence - near UV range; auramine rhodamine, acridine orange and calco-fluor white stains**
4. **Electron microscopy - can resolve particles 0.001 µm apart; useful in viral I.D.**

CULTURE CONDITIONS

1. **Most plates incubated at 35-37°C**
    a. ***Campylobacter* - 42°C**
    b. ***Yersinia* - 25-30°C**
    c. **5-10% CO_2 (*Campy- microaerophilic*)**
    d. **50-70% humidity**
2. **Anaerobic Conditions**
    a. **Broths with thioglycollate or cysteine**
    b. **Pre-reduced media**
        - ❖ ***Gas pak jars or anaerobic chamber***
        - ❖ ***Environment: 10% H_2, 5% CO_2, 85% N_2, palladium crystals***

MEDIA

1. **Approximately 1.5% agar**
2. **Non-selective**
    a. **Supports most organisms**
    b. **Blood agar, chocolate agar, trypticase soy agar**
3. **Selective agar**
    a. **Contains chemicals, dyes, antibiotics to inhibit certain organisms (*EMB, MAC, CNA, Campy-blood*)**
    b. **May also be differential (*HE, SS, XLD, EMB, MAC*)**

INOCULATION

1. Streak for isolation with nichrome or platinum, or disposable loops
2. Calibrated 0.01 ml or 0.001 ml (*.001 ml for urine colony count plates)*
3. Number of colonies x 100 *(.01 loop)* or number of colonies x 1000 *(.001 loop)*
4. Read and report after 18-24 hrs

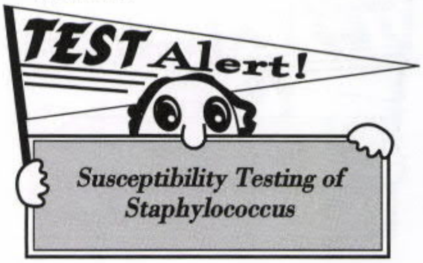
Stains Commonly Used in Microbiology/Mycobacteriology

	PRIMARY STAIN	DECOLORIZER	COUNTERSTAIN	RESULTS		PRINCIPLE
				POS	NEG	
Gram Stain	Crystal Violet	Alcohol/Acetone	Safranin	Purple	Pink	• Iodine mordant • Methanol or heat fix • Violet dye & iodine form complex in cell; washes out of gram neg cells
Kinyoun & Ziehl-Nielson	Carbol Fuchsin	Acid Alcohol	Methylene Blue	Pink	Blue	• Acid fast • For *Mycobacteria*
Auramine-Rhodamine	Auramine and Rhodamine *(Fluorescent Stain)*	Acid Alcohol	Potassium Permanganate	Orange Fluoresc.	No Fluoresc.	• For *Mycobacteria*
Calcofluor White	Calcofluor White + 10% KOH			Bluish-white Fluoresc.	No Fluoresc.	• For yeast and fungi • KOH to break down debris and mucous

Gram Positive Cocci

STAPHYLOCOCCUS

1. "Grape-like" clusters
2. *S. aureus*
   a. Coagulase positive
   b. Most common pathogen of genus
   c. Common infections
      ❖ *Furuncles (boils) and carbuncles*
      ❖ *Bullous impetigo (blisters)*
      ❖ *Paronychia (nails)*
      ❖ *Post surgical wounds and bacteremia*
   d. Intoxications
      ❖ *Scalded skin syndrome (exfoliatin - neonates)*
      ❖ *Toxic shock syndrome (TSST-1) - women ages 12-52*
      ❖ *Food poisoning (enterotoxin) - symptoms in 1-5 hrs after ingestion (potato salad, cream dishes)*
   e. Exotoxins - hemolysins, leukocidins, coagulase and hyaluronidase *(spreading factor)*, nuclease, protease and lipase
   f. Resistance/sensitivity
      ❖ *Most resistant to penicillin due to plasmid mediated B-lactamase*
      ❖ *Some sensitive to penicillinase-resistant penicillins (PRP's) (methicillin, oxacillin, etc); if methicillin-resistant S. aureus (MRSA), vancomycin is drug of choice*
   g. Laboratory diagnosis
      ❖ *BAP - soft, opaque, regular colonies 2-3 mm in diam; some are beta hemolytic and some have pale golden color*
      ❖ *Growth in 7.5% NaCl and ferment mannitol*
      ❖ *Catalase positive and coagulase positive*
      ❖ *Pulse Field Gel Electrophoresis (PFGE) and susceptibility profile for epidemiologic studies*
3. Coagulase negative *Staphylococcus*
   a. Opportunist in immunocompromised hosts and patients with prosthetic valves and devices



Biochemical Tests

CATALASE TEST

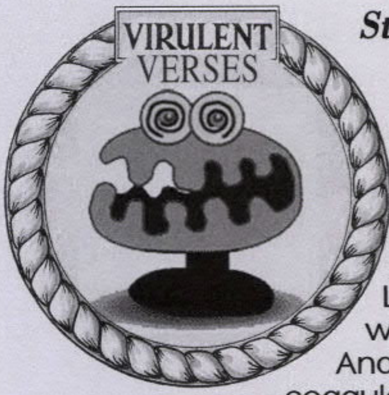
1. Reagent: 3% H_2O_2
2. Add one drop to colony on slide
3. If catalase present, H_2O_2 is broken down to water and O_2 *(which bubbles off)*
4. Positive: *Staph*; negative - *Strep*
5. QC each day of use

COAGULASE TEST

1. Reagent - EDTA rabbit plasma
2. Bound coagulase - clumping on slide *(plasma and colony)*
3. Free coagulase - gels in tube test *(0.5 ml plasma and colony; 35-37°C 4-12 hrs)*
4. Agglutination tests - detect coagulase and protein A
5. Positive: *S. aureus*; negative: other Staph *(human pathogens)*

Staphylococcus (Catalase Positive)

ORGANISM	COAGULASE	INFECTIONS	INTOXICATIONS	NOTES
S. aureus	+	Carbuncles, furuncles, paronychia, wounds, and bacteremia	Scalded Skin Syndrome, Toxic Shock Syndrome, and Gastritis *(enterotoxin, 1-5 hours after eating)*	Most beta lactamase +; many *MRSA* CA= community acquired HA=hosptial aquired
S. epidermidis	–	Endocarditis, prosthetic device infections		Most methicillin resistant; sensitive to novobiocin
S. saprophyticus	–	UTI in young women		Resistant to novobiocin
Coag Negative Staph: Opportunistic Infections in Immunocompromised Patients and Those with Prosthetic Devices				



Staphylococcus aureus

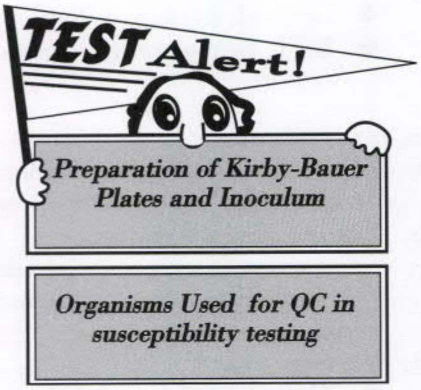
Staph aureus is
my nickname
In the
pathogen Hall
of Fame
Look for bubbles
with catalase
And for clots with
coagulase.

Gram positive clusters everywhere
Skin and gut, nose and hair.

Protein A is my main trait
and Enterotoxin in what you ate.

Penicillin won't touch me
Methicillin, well . . . maybe?
Vancomycin killed in days of yore
But now that drug is not for sure.

Wash your hands, don't pick your nose.
Wear a lab coat over your clothes.



Preparation of Kirby-Bauer Plates and Inoculum

Organisms Used for QC in susceptibility testing

STREPTOCOCCUS

1. Spherical or oval; chains or pairs
2. Lancefield grouping based on C carbohydrate
3. *S. pyogenes (Group A)*
   - a. Beta hemolytic
   - b. Streptolysin S - stable in O_2; non-antigenic
   - c. Streptolysin O - oxygen labile; antigenic
   - d. Erythrogenic toxin - rash of scarlet fever

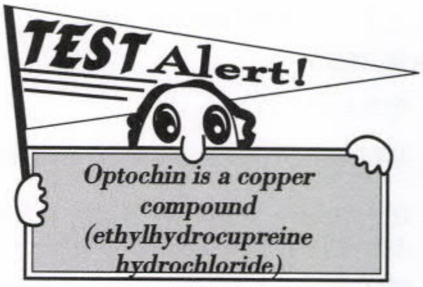
- e. Highly sensitive to penicillin
- f. Infections
  - ❖ *Pharyngitis (Strep throat)*
  - ❖ *Impetigo*
  - ❖ *Erysipelas*
  - ❖ *Wounds, burns*
  - ❖ *Rheumatic fever (autoimmune sequelae to infection with Streptococcus group A)*
  - ❖ *Lab diagnosis*
    - ☞ Sensitive to 0.04 units bacitracin disc
    - ☞ Typing

4. *S. agalactiae (Group B)*
   - a. Narrow zone of beta hemolysis
   - b. Neonatal sepsis and meningitis; UTI; vaginal infections
   - c. Laboratory diagnosis
     - ❖ *Serotyping*
     - ❖ *CAMP reaction (with S. aureus)*
     - ❖ *Na hippurate positive*

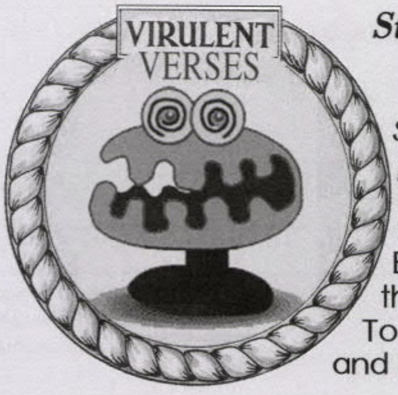
5. Group D
   - a. *S. bovis/ gallolyticus*
     - ❖ *BEM positive*
     - ❖ *No growth in 6.5% NaCl*
     - ❖ *Associated with colorectal cancer*
   - b. *Enterococcus*
     - ❖ *Bile esculin medium (BEM) positive*
     - ❖ Growth in 6.5% NaCl
     - ❖ UTI, bacteremia, others

6. *S. pneumoniae*
   - a. Alpha hemolytic crater-like colonies or mucoid, "water drop" colonies
   - b. Lancet-shaped diplococci
   - c. Check sensitivity to penicillin using OX (oxacillin) disc (*≥ 20 mm = sensitive)*
   - d. Causes
     - ❖ *Primary lobar pneumonia (rusty sputum)*
     - ❖ *Meningitis*
     - ❖ *Bacteremia*
     - ❖ *Otitis media*
     - ❖ *Conjunctivitis*
   - e. Laboratory diagnosis
     - ❖ *Typical colony morphology*
     - ❖ *Quellung reaction*
     - ❖ *Sensitive to optochin*
     - ❖ *Bile soluble*

7. Other alpha *Streptococcus* (*viridans group*) - subacute bacterial endocarditis (SBE)



Optochin is a copper compound (ethylhydrocupreine hydrochloride)



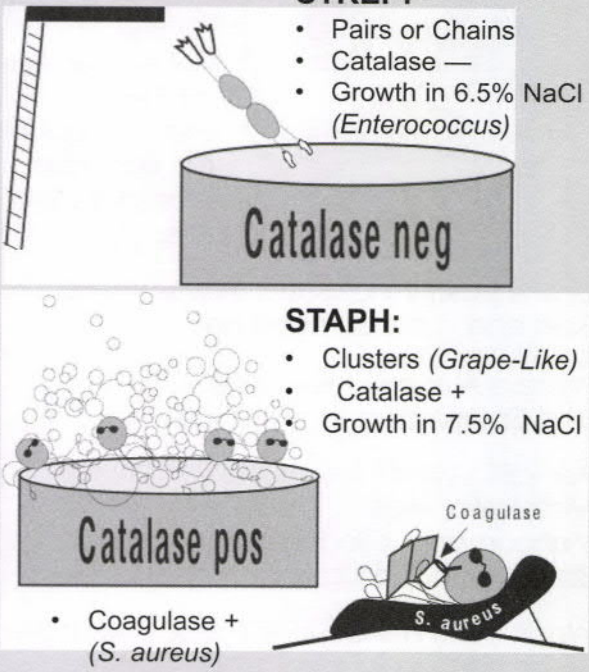
Streptococcus pyogenes

Streptococcus pyogenes
Penicillin will kill
with ease.
But Protein M is
the biggest trick
To make throat
and muscle sick.

Antistreptolysin O
Will lay the heart and kidneys low.
Bacitracin is my sign
Zone of inhibition every time.

See what I do on B-A-P
Destroy the sheep R-B-C.

REMEMBER!
Staph and Strep



STREP:

- Pairs or Chains
- Catalase —
- Growth in 6.5% NaCl *(Enterococcus)*

STAPH:

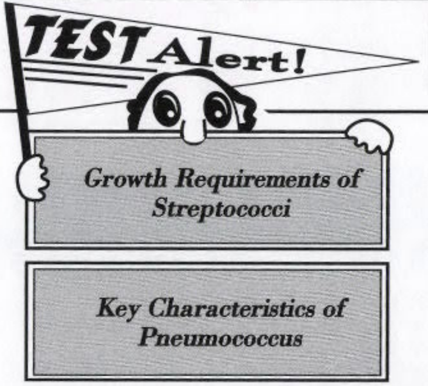
- Clusters *(Grape-Like)*
- Catalase +
- Growth in 7.5% NaCl
- Coagulase + *(S. aureus)*

(*See Streptococci Chart on next page.*)

Streptococci (Catalase Negative)

	Hemolysis	Bacitracin	Na Hippurate	Optochin	Bile Solubility	Bile Esculin	6.5% NaCl	Infections
S. pyogenes (group A)	β	S	–	R	–	–	–	Pharyngitis, wounds, scarlet fever, impetigo, sequalae-rheumatic fever
S. agalactiae (group B)	β, γ	R	+	R	–	–	–	Neonatal septicemia and meningitis, UTI *(CAMP +)*
Entercoccus (group D)	α, β, γ	R	–	R	–	+	+	UTI, endocarditis *(treat with aminoglycoside+penicillin)*
Non-enterococcus (group D)	α, γ	R	–	R	–	+	–	Endocarditis *(rare), Strep. bovis associated with color cancer*
S. pneumoniae	α *("water drop"/ crater colonies)*	R	–	S	+	–	–	Pneumonia, meningitis, bacteremia *(screen for penicillin sensitive with Ox; > 20 mm = sensitive)*
S. viridans	α, γ	R	–	R	–	–	–	Endocarditis *(rare)*

α hemolysis = greening around colony
δ hemolysis = no hemolysis
β hemolysis = complete clearing around colony
Shaded areas = Key Reactions



Growth Requirements of Streptococci

Key Characteristics of Pneumococcus

REMEMBER!

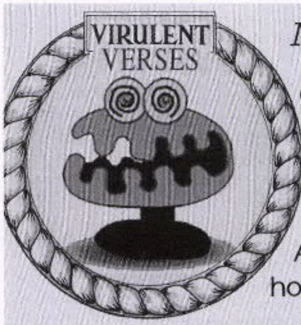
Victory Belongs to the Most Persevering.

Gram Negative Cocci

NEISSERIA AND MORAXELLA

1. Key characteristics
   a. Diplococci (*kidney bean shape*)
   b. Oxidase positive

2. *N. gonorrhoeae*
   a. Grows on chocolate and Thayer-Martin (*contains vancomycin, colistin, nystatin, hemoglobin and isovitalex*)
   b. Requires 5-10% CO_2; may take 48 hrs for growth
   c. Ferments glucose
   d. Gonorrhea
      - ❖ *Sexually transmitted*
      - ❖ *May be asymptomatic; may be mixed with Chlamydia*
      - ❖ *Gram stain sensitive for males, but NOT for females*
      - ❖ *May be confused with Moraxella or Acinetobacter*
      - ❖ *Do NOT refrigerate prior to culture*
      - ❖ *Penicillin or spectinomycin sensitivity; perform beta lactamase test to determine penicillin sensitivity*
   e. Most ID by probe technology



Neisseria gonorrhoeae

Gonorrhea, the clap, GC
I'm an old-fashioned STD.

I need CO_2 and chocolate agar
And may not grow for 48 hours.

I ferment glucose, the only sugar for me,
Do an oxidase and purple you'll see.

3. *N. meningitidis*
   a. Grows on blood agar, chocolate and Thayer-Martin
   b. 5-10% CO_2 enhances growth
   c. Ferments glucose and maltose
   d. Transmitted by respiratory droplets and requires close contact
   e. Meningitis
      - ❖ *Seen mostly in children under 3*
      - ❖ *Waterhouse - Friderichsen syndrome (scattered petechiae) = meningiococcemia*
      - ❖ *Mainly caused by types A, B, C, Y & W*

4. Bacterial meningitis- CSF
   a. ↑neutrophils
   b. ↓glucose
   c. ↑protein

5. *Moraxella* (*Branhamella*) *catarrhalis*
   a. Respiratory infections
   b. Grows well on chocolate and BAP but not on MacConkey's
   c. Colony hard and "moves over"; asaccharolytic
   d. Usually beta lactamase positive
   e. DNAse positive

6. *Acinetobacter* species
   a. Emerging pathogen
   b. Respiratory infections, UTI, or colonizer
   c. Coccobacillus
   d. Resistance to many drugs; AST required
   e. Identification
      - ❖ *Growth on MAC*
      - ❖ *Some hemolytic*
      - ❖ *Oxidase negative*

REMEMBER!

Differentiating Neisseria

	Glucose	Maltose	Lactose
N. gonorrhoeae	+	—	—
N. meningitidis	+	+	—
N. lactamica	+	+	+

All ferment glucose; *gonorrhoeae*: only glucose; *meningitidis*: glucose and maltose; and *lactamica*: glucose, maltose and lactose.

Gram Positive Rods

CORYNEBACTERIUM DIPHTHERIAE

1. Key Characteristics
   a. Small pleomorphic rods with clubbed ends
   b. Palisade or "chinese letter" arrangement
   c. Metachromatic granules (*stain red-purple with methylene blue*)
   d. Tinsdale agar - black colonies due to tellurite hydrolysis
   e. Elek test - determines toxin production by the isolate in vitro

2. Loeffler's - enhances development of metachromatic granules
   a. Pallisade arrangement may be seen

3. **Exotoxin production by only lysogenic organsims carrying a B phage**

4. **Produces pseudomembrane on tonsils, uvula or soft palate**

5. **Causes diphtheria**

GARDNERELLA VAGINALIS

1. **Bacterial vaginosis**

2. **"Clue cells" - high number of squamous epithelial cells colonized with gram variable rods**

3. **10% KOH added to discharge causes "fishy" odor**

4. **Tiny colonies at 48 hrs on BAP and chocolate**

5. **Catalase and oxidase negative; hippurate and starch positive**

LISTERIA MONOCYTOGENES

1. **Key Characteristics**
   a. **Small colonies with narrow zone of beta hemolysis**
   b. **Catalase positive**
   c. **Tumbling motility; "umbrella" motility in SIM at room temperature but NOT 37°C**
   d. **Bile esculin positive**

2. **Causes neonatal meningitis and sepsis; sepsis in immunocompromised hosts**

ERYSIPELOTHRIX

1. **Key Characteristics**
   a. **Non-motile**
   b. **Catalase negative**
   c. **"Test tube brush" growth in gelatin; H_2S positive in TSI**

2. **Occupational infection for fishermen, butchers, veterinarians, rose growers**

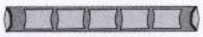
BACILLUS SP. *(Sporeformers)*

1. **Key Characteristics**
   a. **Large, ground glass colonies**
   b. **Some beta hemolysis (*But not B. anthracis)***
   c. **Catalase positive**
   d. **Large gram positive to variable rods in chains with spores**

2. ***B. anthracis***
   a. **Very long chains (*"bamboo shoots"*)**
   b. **"Medusa head" colonies**
   c. **Non-motile, non-hemolytic**
   d. **Anthrax - cutaneous, pulmonary, or gastrointestinal**

3. ***B. cereus*** **- Food poisoning due to preformed toxin - "fried rice"**

Gram Positive Rods

ORGANISM	CATALASE	ESCULIN	H_2S /TSI	β HEMOLYSIS	NOTES
Corynebacterium 	+	—	—	+/—	"Chinese Letter" Arrangement; Metachromatic Granules *(Loeffler's Slants)*; Tellurite Hydrolysis *(Tinsdale Agar)*; Elek Test Determines Toxin Production
Listeria 	+	+	—	+	Tumbling Motility at 25° C, but Not 37C; Cold Enrichment; Neonatal Meningitis and Sepsis; Sepsis in Immunocompromised Hosts
Erysipelothrix 	—	—	+	—	"Test Tube Brush" Growth in Gelatin; Infection in Fishermen, Butchers, Veterinarians
Bacillus *(Spore Formers)* 	+	V	—	— / +	"Ground Glass" Hemolytic Colonies; • *B. anthracis* -Non-Hemolytic, Non-Motile, "Medusa-head" colonies (filamentous outgrowth) • *B. cereus*- betahemolytic, motile, large flat colonies (irregular edges)

Gram Negative Rods

ENTEROBACTERIACEAE

1. General Characteristics
   a. Peritrichous flagella when motile
   b. Ferment glucose
   c. Reduce NO_3 to NO_2
   d. Most oxidase negative
   e. Antigens used in typing:
      - ❖ *Flagella = H Ag*
      - ❖ *Envelope = K Ag*
      - ❖ *Cell wall LPS (lipopolysaccharide) = O Ag*
      - ❖ *All possess LPS endotoxin; some produce exotoxins*

Escherichia coli

1. Key Characteristics
   a. Indole and lactose positive
   b. IMViC = ++ - -

2. Most common cause of UTI in females

3. Intestinal infections
   a. Enterotoxigenic *E. coli* (*ETEC*)
      - ❖ *LT toxin (heat labile)*
      - ❖ *ST toxin (heat stable)*
   b Enteroinvasive *E. coli (EIEC)*
      - ❖ *Penetrate epithelial cells in large intestine*
      - ❖ *May be lactose negative*
   c. Enterohemorrhagic *E. coli* (*EHEC*) - strain 0157:H7
      - ❖ *Shigella-like toxin*
      - ❖ *Food poisoning associated with undercooked meat (hamburger)*
      - ❖ *Hemolytic uremic syndrome (HUS)*

4. K1 strains can cause neonatal meningitis

Shigella

1. Key Characteristics
   a. Lactose negative
   b. Non-motile
   c. Anaerogenic

2. Bacillary dysentery - penetrate epithelial cells in small intestine

3. *S. dysenteriae (Group A)* - most severe

4. *S. flexneri (Group B)*

5. *S. boydii (Group C)*

6. *S. sonnei (Group D)* - most common

7. < 200 organisms needed for disease

REMEMBER!
Shigella

S. dysenteriae = Group A *(1st alphabetically)*
S. flexneri = Group B *(not "B"oydii)*
S. boydii = Group C *("boyd ee ee" = "C")*
S. sonnei = Group D *(last alphabetically)*

Common Gram Negative Selective Media

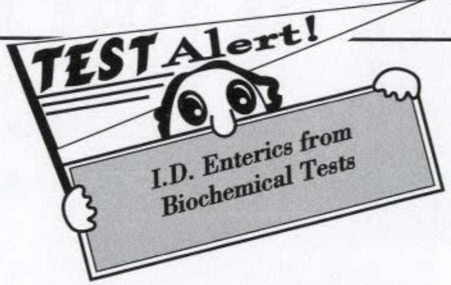
AGAR	DIFFERENTIATING AGENT	SELECTIVE AGENT	H_2S INDICATOR	LACTOSE POSITIVE	LACTOSE NEGATIVE
MAC MacConkey	Lactose	Crystal Violet, Bile Salts	None	Dark Pink	Transparent
EMB *(Eosin, Methylene Blue)*	Sucrose Lactose	Eosin Y Methylene Blue	None	Green Sheen, Purplish and Brownish Amber	Transparent
HE *(Hektoen-Enteric)*	Lactose Sucrose Salicin	Bile Salts	Sodium Thiosulfate	Salmon	Green to Blue *(H_2S+ = Black)*
SS *(Salmonella - Shigella)*	Lactose	Brilliant Green Bile Salts	Sodium Thiosulfate	Red	Transparent *(H_2S+ = Black Center)*
XLD *(Xylose - Lysine Deoxycholate)*	Lactose Sucrose Xylose	Bile Salts	Sodium Thiosulfate	Yellow	Transparent on Red Medium *(H_2S+ = Black)*
Enrichment Broths = Selenite and GN Broth					

Enterobacteriaceae: Primary Differentiating Tests

	TSI	Ornithine	VP	Urease	Lysine	Motility	H_2S	Deaminase	DNAase
Citrobacter	K(A)/A, Gas	v	–	v	–	+	v	–	–
Enterobacter	A/A, Gas	+	+	–	v	+	–	–	–
Escherichia	A(K)/A, Gas	v	–	–	+	v	–	–	–
Klebsiella	A/A, Gas	–	+	v	+	–	–	–	–
Morganella	K/A, Gas	+	–	+	v	+	–	+	–
Proteus	K/A, Gas	v	–	+	–	+	+	+	v
Providencia	K/A, Gas	–	–	v	–	+	–	+	–
Salmonella	K/A, Gas	+	–	–	+	+	+	–	–
Serratia	K(A)/A	v	+	–	+	+	–	–	+
Shigella	K/A	v	–	–	–	–	–	–	–
*Plesiomonas**	K/A	+	–	–	+	+	–	–	–

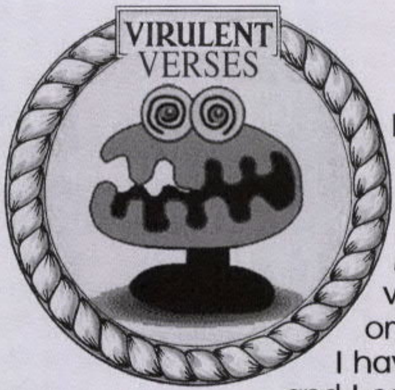
* oxidase positive

v = variable
Shaded areas = Key reactions differentiating similar genera


TEST Alert!
I.D. Enterics from Biochemical Tests

Klebsiellae

1. Opportunist; UTI, pneumonia; ampicillin-resistant
2. *Klebsiella*
    a. Non-motile
    b. Has capsule
    c. Urea variable
    d. Ornithine negative
    e. VP positive
    f. Can cause lobar pneumonia
    g. New member: *K. granulomatis*
        ❖ Formerly *Calymanatobacterium*
        ❖ STI
3. *Enterobacter*
    a. Motile
    b. Ornithine positive
4. *Serratia*
    a. May produce red pigment
    b. DNase, gelatinase positive
    c. VP positive



Klebsiella pneumoniae

Kleb pneumo in
lungs, where
else would I
be?
Maybe in a
wound, bladder
or kidney.
I have no flagella
and I can't swim.
Test this out with S-I-M.
I'm blue on citrate, prefer lysine.
Maybe pink on urea, hate ornithine.

Salmonella

1. Large number needed for infection *(> 100,000)*
2. Biochemical reactions
    a. H_2S positive
    b. Lysine positive
    c. Indole negative
    d. Urea negative
3. *S. enterica*- >2000 serotypes including
    a. *S. cholerasuis* - septicemia
    b. *S. typhi* - typhoid fever
        ❖ *Blood positive early - 1st week*
        ❖ *Stool positive late- 2nd-3rd week*
    c. *S. arizone* - ONPG pos *(others neg)*

Citrobacter

1. Opportunist
2. Lysine negative
3. Similar to *Salmonella* biochemically

Proteus

1. Key Characteristics
    a. Urea positive
    b. Deaminase positive
2. *P. mirabilis*
    a. Most sensitive to penicillins
    b. Indole negative
3. *P. vulgaris*
    a. Indole positive
    b. H_2S positive

Yersinia

1. *Y. enterocolitica*
    a. Optimal growth = RT; cold enrichment
    b. Invasive and toxigenic
2. *Y. pseudotuberculosis* - Acute mesenteric lymphadenitis and "pseudotubercules"
3. *Y. pestis* - bubonic plague

Plesiomonas

1. Oxidase positive
2. Reclassified into *Enterobacteriaceae*
3. Lophotrichous flagella
4. Associated with diarrheal disease

BIOCHEMICAL TESTS

1. Oxidase test
    a. Reagent tetramethyl p-phenylenediamine dihydrochloride
    b. Positive = purple
2. Nitrate test
    a. Reagents - α-naphthylamine, sulfanilic acid
    b. Positive = pink (*use zinc powder to confirm)*
3. ONPG test
    a. Detects B-d-galactosidase
    b. Reagent - O-nitrophenyl-B-d-galactopyranoside
    c. Positive = yellow
4. TSI (*triple sugar iron agar*) slant
    a. 0.1% glucose, 1% sucrose, 1% lactose
    b. Yellow butt - glucose fermented
    c. Yellow slant - lactose or sucrose fermented

   d. Red slant - neither lactose nor sucrose fermented
   e. Black butt - H_2S produced

5. Kligler Iron Agar (*KIA*) - same as TSI but with only glucose, and lactose, no sucrose

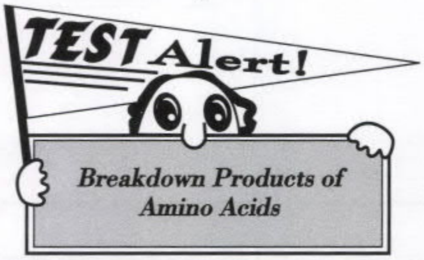
6. Citrate test
   a. Media green
   b. Positive = blue

7. Decarboxylase tests
   a. Measures ability to decarboxylate amino acids
      - ❖ *Lysine ➔ Cadaverine*
      - ❖ *Ornithine ➔ Putrescine*
      - ❖ *Arginine ➔ Putrescine*
      - ❖ *Indicator dye = bromcresol purple*
   b. Lysine iron agar (LIA)
      - ❖ *Has H_2S indicator*
      - ❖ *0.1% glucose and 1% lysine*
      - ❖ *Positive = purple butt*
      - ❖ *Slant of LIA turns red for lysine deaminase*
   c. Motility-indole-ornithine (MIO)
      - ❖ *0.1% glucose and 1% ornithine*
      - ❖ *Positive = purple butt*
      - ❖ *Also tests for motility and indole (Kovac's)*

8. Indole test
   a. Indole split from tryptophan
   b. Reagent (*Kovac's*) - p-dimethyl-aminobenzaldehyde
   c. Positive = pink

9. Urease test
   a. Urea hydolyzed to ammonia and CO_2
   b. Phenol red indicator turns pink if positive

10. Voges-Proskauer (*VP*)
   a. Detects acetylmethylcarbinol (*acetoin*)
   b. Reagents = KOH and α-naphthol
   c. Positive = pink

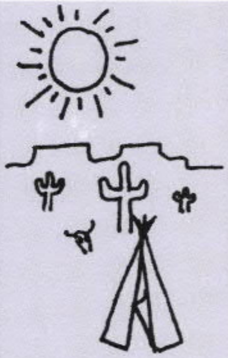


Breakdown Products of Amino Acids

REMEMBER! *Enterobacteriaceae* (TSI Reactions)

Picture yourself in a desert.
The yellow sun shining over the hot desert = A/A.
The moon shining over the desert = K/A.
The moon over the mountain = K/K.

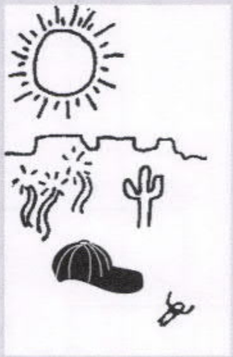
A/A
(Sun over Desert)



S erratia
E scherichia
E nterobacter
K lebsiella

Seek shelter in the hot desert.

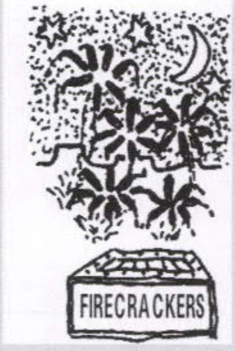
A/A, H_2S +
(Sun over Desert)



C itrobacter
A rizona
P roteus

Wear a cap to protect yourself from the geyser (H_2S +).

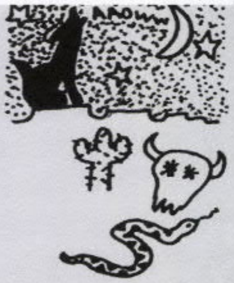
K/A, H_2S +
(Moon over Desert)



C itrobacter
A rizona
S almonella
E dwardsiella

A case of fire-crackers going off smells like sulphur. (H_2S +).

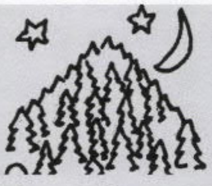
K/A
(Moon over Desert)



S higella
Ci trobacter
P rovidencia
P lesiomonas
P roteus
Y ersinia

Sk(c)ipppy Coyote howls at the moon.

K/K
(Moon over Mountain)

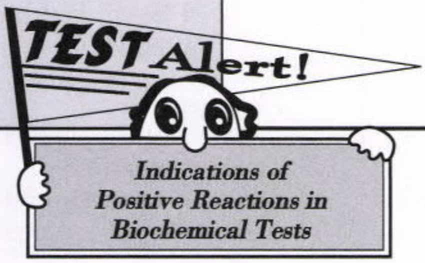


P seudomonas

Pseudo"moon"as

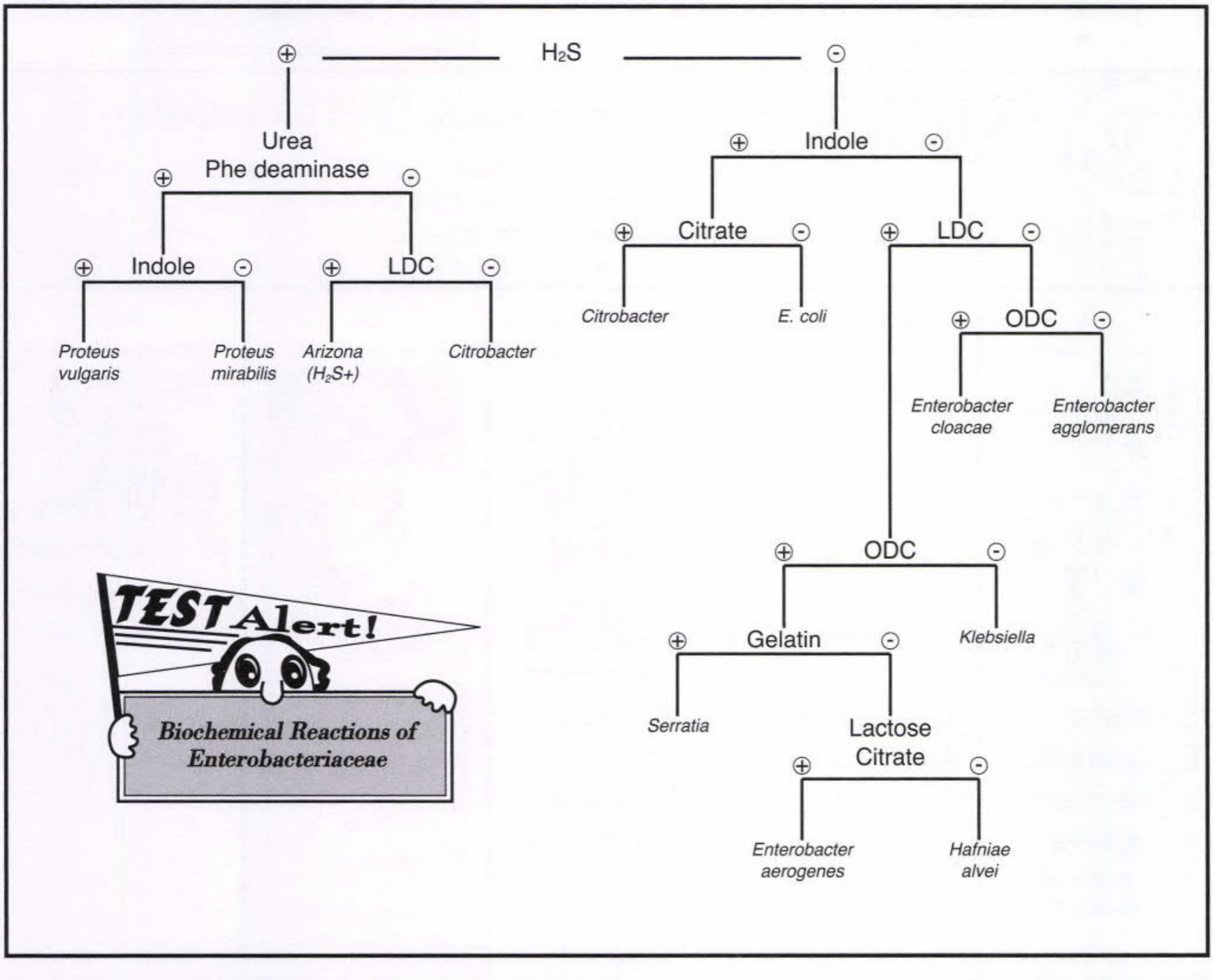
TSI - Biochemical Reactions

A/A	A/A, H_2S+	K/A, H_2S+	K/A	K/K
Escherichia *(indole+)*	*Proteus* *(urea+, deam+)*	*Salmonella* *(mal-, ONPG-)*	*Shigella* *(citrate-, non-motile)*	*Pseudomonas* *(ox+, blue-green pigment, growth at 42°C, growth in cetrimide)*
Enterobacter *(ODC+, sugars)*	*Arizona* *(LDC+)*	*Citrobacter* *(LDC-)*	*Providencia* *(deam+)*	*Other non-fermenters*
Klebsiella *(ODC-)*	*Citrobacter* *(LDC-)*	*Edwardsiella* *(indole+, LDC+)*	*Citrobacter* *(citrate+)*	
Serratia *(sugars)*			*Plesiomonas* *(oxidase +)*	
			Proteus *(urea+, deam+)*	
			Yersinia *(small colonies, urea+, deam-)*	



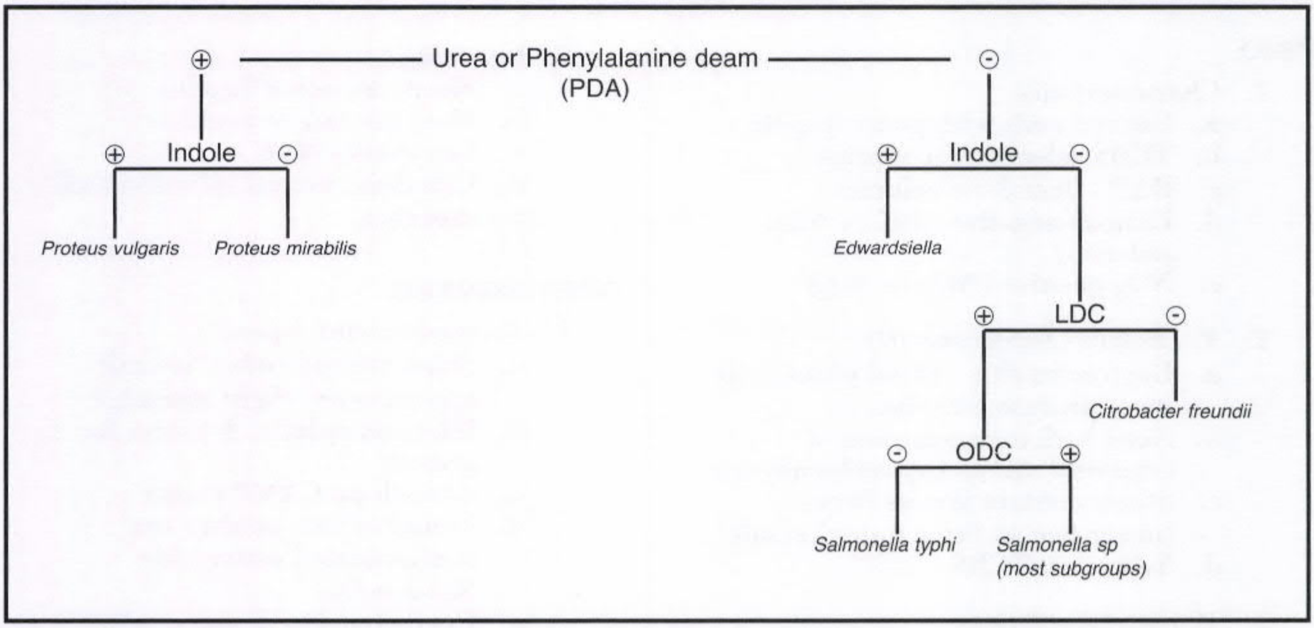
Indications of Positive Reactions in Biochemical Tests

Enterobacteriaceae - A/A, Gas


⊕ H2S ⊖
Urea
Phe deaminase
⊕ ⊖
⊕ Indole ⊖
Proteus vulgaris
Proteus mirabilis
⊕ LDC ⊖
Arizona (H2S+)
Citrobacter
⊕ Indole ⊖
⊕ Citrate ⊖
Citrobacter
E. coli
⊕ LDC ⊖
⊕ ODC ⊖
Enterobacter cloacae
Enterobacter agglomerans
⊕ ODC ⊖
⊕ Gelatin ⊖
Klebsiella
Serratia
Lactose
Citrate
⊕ ⊖
Enterobacter aerogenes
Hafniae alvei
TEST Alert!

Biochemical Reactions of Enterobacteriaceae

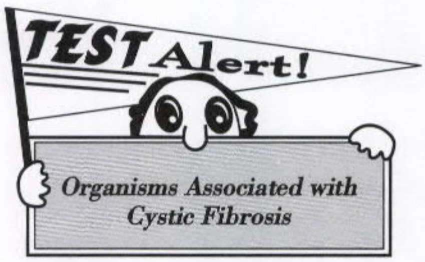
Enterobacteriaceae - K/A, H_2S+


⊕ Urea or Phenylalanine deam (PDA) ⊝
⊕ Indole ⊝
Proteus vulgaris
Proteus mirabilis
⊕ Indole ⊝
Edwardsiella
⊕ LDC ⊝
Citrobacter freundii
⊝ ODC ⊕
Salmonella typhi
Salmonella sp (most subgroups)

11. Phenylalanine deaminase
    a. Reagent = ferric chloride
    b. Positive = green

GRAM NEGATIVE NON-FERMENTERS

1. Oxidase- positive (*some exceptions, i.e. Stenotrophomonas*)
2. May not grow on MacConkey's agar
3. Glucose NOT fermented
    a. TSI= alk/alk
    b. Oxidative Fermentative (OF) media
        - ❖ Semi-solid
        - ❖ Low protein
        - ❖ Bromthymol blue indicator
        - ❖ Incubate tubes in pairs
            - ☞ *Open- oxygen*
            - ☞ *Closed - oxygen-restricted*
            - ☞ *Pair of tubes yellow = fermenter*
            - ☞ *Pair of tubes green = asaccharolytic (non-utilizer)*
            - ☞ *Open tube yellow and closed tube green = oxidizer*
4. *Pseudomonas aeruginosa*
    a. Most common
    b. Oxidase positive
    c. Growth on MAC (*lactose negative*)
    d. Oxidizer *(OF)*
    e. Polymyxin B susceptible
    f. Opportunistic pathogen
        - ❖ *Mucoid in CF patients*
        - ❖ *Pigment production*
            - ☞ *Pyocyanin (blue green)*
            - ☞ *Pyorubin (rust)*
            - ☞ *Pyoverdin (fluorescent)*
    g. Drug resistant
    h. Infections- burns, pneumonia, swimmer's ear, eye infections, UTI
5. *Burkholderia cepacia*
    a. Oxidase weakly positive
    b. Growth on MAC
    c. Oxidizer (*OF*)
    d. Polymyxin B resistant
    e. Opportunistic pathogen
        - ❖ *Serious infection in CF patients*
        - ❖ *Yellow-brown insoluble pigment*



Organisms Associated with Cystic Fibrosis

Gram Negative
Oxidase Positive Fermenters

VIBRIO

1. Characteristics
   a. Curved rods with polar flagella
   b. TCBS selective for vibrios
   c. BAP - hemolytic colonies
   d. Lactose negative (*differs from enterics)*
   e. NO_3 positive *(NO_3 to NO_2)*

2. *V. cholera* (*Serogroup 01*)
   a. Gastroenteritis - rapid onset 3-10 hrs.; profuse diarrhea
   b. Need high concentration of organism unless hypochlorohydric
   c. Stools contain mucus flecks (*described as "rice water" stools)*
   d. Yellow on TCBS

3. *V. parahemolyticus*
   a. Green on TCBS
   b. Enteritis

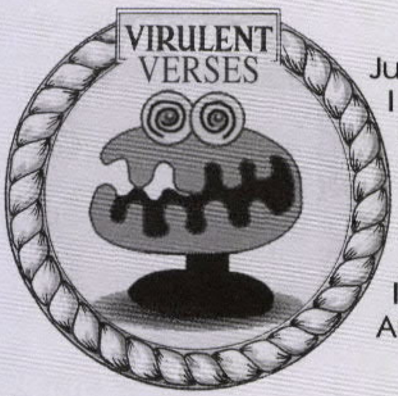
4. *V. vulnificus*
   a. Green on TCBS
   b. Septicemia - can kill immunocompromised or diabetics

5. *Aeromonas*
   a. Motile by polar flagella
   b. Most are indole positive
   c. Growth on MAC
   d. Cellulitis, wound infections and diarrhea

OTHER ORGANISMS

1. *Campylobacter jejuni*
   a. Small curved rods, "seagull appearance" *(light staining)*
   b. Microaerophilic, 2-4 days for growth
   c. Growth on CAMPY agar
   d. Found in raw poultry and contaminated water (*like Salmonella*)
   e. Erythromycin or tetracycline for treatment
   f. Biochemical reactions
      ❖ *Catalase positive*
      ❖ *Oxidase positive*
      ❖ *Hippurate positive*

2. *Helicobacter pylori*
   a. Associated with gastric and duodenal ulcers
   b. Produces large amounts of urease



Campylobacter jejuni

Just call me Jay June Eye.
I might be in water or chicken pot pie.
I like just a touch of 0_2
More oxygen and I'll be through!

Campy-plates are best to grow
At 42 degrees or so
If I grow, Gram stain me
And curved rods are what you'll see.

Zoonotic Diseases (Acquired Directly or Indirectly from Animals)

ORGANISM	DESCRIPTION	DISEASE	NOTES
Brucella	Gram Neg Coccobacillus	Brucellosis	Blood Culture Pos in First Two Weeks *(Hold 21 days)*

	CO_2	**H_2S**	**Thionine**	**Basic Fuschin**
B. abortus **(cows)**	+	+	+	—
B. suis **(pigs)**	—	+/—	—	+
B. melentensis **(goats)**	—	—	—	—

REMEMBER! Brucella

Cows eat Thionine.

Pigs eat Basic Fuschin.

Goats eat neither.

ORGANISM	DESCRIPTION	DISEASE	NOTES
Francisella tularensis **Francis the Rabbit Uses the Hood** 	Faintly staining Gram Neg Coccobacillus Tiny Pinpoint Colonies Cystine-Glucose Media H2S + with Lead Acetate	Tularemia "Rabbit Fever"	Infected by Tick Bite HIgh Risk to Lab Personnel Biosafety level 3
Yersinia pestis **Yersin the Safety Pin** 	Gram Neg Bi-Polar Staining *(resembles safety pin)*	Plague	Transmitted by Fleas, Rats, Other Mammal Reservoirs Biosafety level 3
Pasteurella multocida 	Gram Neg Rod Bi-Polar Staining Oxidase and Indole Positive Ferments Glucose and Sucrose	Contracted from Cat and Dog Bites	"Mousy" Odor Biosafety level 2
Streptobacillus moniliformis 	Long Filamentous Gram Neg Rods with Swellings (*pleomorphic*); "Puffball" or "String of Pearls" Colonies in Thioglycolate Broth	"Rat Bite Fever" Haverhill Fever	Ascitic Fluid Sample Needed; Sodium Polyanethol Sulfonate (*SPS*) Inhibits growth

Fastidious Gram Negative Rods

GENERAL INFORMATION

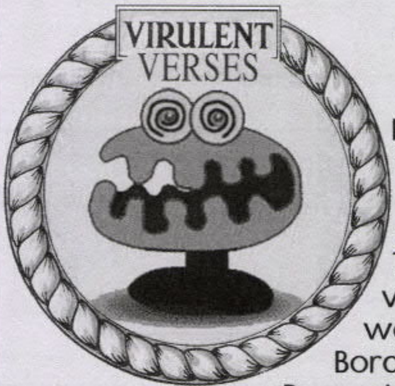
1. Source - mouth flora
2. Pathogenic in immunocompromised hosts; causes:
   a. Peridontal and jaw abscesses (*Eikenella, Aggregatibacter, Capnocytophaga*)
   b. Infectious endocarditis
   c. Bacteremia (*Capnocytophaga, Cardiobacterium*)
3. Grow slowly and require 5 - 10% CO_2; NO growth on MAC

CAPNOCYTOPHAGA

1. Capnophilic
2. Fusiform shape
3. Bacteremia/ septicemia
4. Gliding motility

BORDETELLA PERTUSSIS

1. Gram negative coccobacilli; causes "whooping cough"
2. Require special media
   a. Classic = Bordet-Gengou (*potato infusion with glycerol and 20% SRBc's)* and penicillin
   b. Regan/ Lowe (*oxoid charcoal agar, 10% horse blood, cephalexin*); longer shelf life
3. Old method for collection - cough plate; better to collect NP swab and plate directly; PCR to confirm ID
4. Colony
   a. Incubate 72-96 hrs, 35°C
   b. Pinpoint, "mercury droplet" colonies



Bordetella pertussis

Pertussis a
fancy name for
whooping
cough.
Take the
vaccine to be
well off.
Bordet-Gengou or
Regan Lowe
Are the best for me to grow.
It may take many days to see
A mercury droplet colony.

HAEMOPHILUS INFLUENZAE

1. Small, non-motile gram negative rod
2. Requires growth factors
   a. X factor = hemin
   b. V factor = NAD
   c. Both factors found in blood, but need heat to break down red cells and release factors, so chocolate used
   d. Satellitism
      ❖ *S. aureus produces V factor and releases X factor by hemolyzing blood*
      ❖ *Haemophilus will grow in the hemolytic zone surrounding staph as satellite colonies on blood agar plate*
      ❖ *Need 5% horse or rabbit blood to see hemolysis*
   e. Infections
      ❖ *Meningitis -*
         ☞ 2-4 years
         ☞ Preceded by nasopharyngeal colonization and bacteremia

HACEK GROUP	OXIDASE	CATALASE	NITRATE	INDOLE	NOTES
Aggregatibacter aphrophilus (Haemophilus)	—/ weak+	—	+	—	Colonies similar to Aggregatbacter; Endocarditis
Aggregatibacter actinomycetemcomitans (Actinobacillus)	+/—	+	+	—	Peridontal & Jaw Abscesses; High Number in Plaque; Center of Colony has 4-6 Pointed Star
Cardiobacterium hominis	+	—	—	+	Can Cause Endocarditis; Can Give False Positive Gram reactions Rosette grouping
Eikenella corrodens	+	—	+	—	"Bleachy" Odor; Pits Agar; Three Zones of Growth; Peridontal and Jaw Abscesses
Kingella kingae	+	—	—	—	Septic Arthiritis; Osteomyelitis in Children; Spreading Corroding Colonies

- ❖ *Epiglottitis*
  - ☞ Ages up to 2 years; do NOT collect throat culture (blood culture best)
  - ☞ May require intubation
- ❖ *Pneumonia <5 years, >60 years*
- ❖ *Conjunctivitis ("pink eye")*
  - ☞ Very contagious
- ❖ *Chancroid - H. ducreyi*
  - ☞ Painful genital ulcers or soft chancres
  - ☞ Gram stain of drainage shows tiny gram negative coccobacilli with a "school of fish" arrangement

3. Characteristics
   a. Growth only on chocolate, NOT blood agar plate (*unless mixed with S. aureus)*
   b. Use X and V discs on Mueller-Hinton agar to detect growth requirements
   c. Susceptibility zone interpretations differ from conventional K- B zones (*use Haemophilus test media (HTM), supplemented Mueller-Hinton agar and incubate in 5-10% CO_2)*
   d. Perform beta lactamase to determine sensitivity to ampicillin; cefuroxime, ceftriaxone, cefotaxime (*meningitis*)

LEGIONELLA PNEUMOPHILA

1. Legionnaires' disease - severe pneumonia; Pontiac fever = milder form

2. 75% illness due to *L. pneumophila* serogroups 1 and 6

3. Sources - potable water, faulty air conditioner vents, lakes and ponds

4. Identification
   a. Specimen - sputum, bronchial washing, pleural fluid, lung aspirate or biopsy
   b. Growth on BCYE (*buffered charcoal yeast extract)* with or without antibiotics but not on chocolate or blood
   c. Direct exam - Giemsa and Gram stain with basic fuchsin counter stain

MYCOPLASMA AND *UREAPLASMA*

1. Smallest free-living microorganisms (125-250 nm)

2. Lack cell wall (*bound by single triple layered membrane*)

3. Does not stain with Gram's stain; can use Dienes stain

4. Center of colony grows into special media (*contains sterols*) giving appearance of inverted "fried egg"

5. *M. pneumoniae*
   a. Primary atypical pneumonia or "walking" pneumonia
   b. Causes positive cold agglutinin titer (*> 1:32*); false positive RPR
   c. Treat with erythromycin or tetracycline

6. *Ureaplasma urealyticum*
   a. Non-gonococcal, non-chlamydial urethritis, especially in males
   b. Produces urease
   c. Treat with tetracycline or spectinomycin

7. *M. hominis*
   a. May colonize GU tract; post partum fever
   b. Tetracycline; resistant to erythromycin (*all other Mycoplasma are sensitive*)

SPIRILLACEAE

1. Rigid, helically curved rods with one or more turns; corkscrew motility by polar flagella; gram negative

2. *Spirillum minor* - "rat bite" fever
   a. Visualize by darkfield or stain with Giemsa
   b. 2-3 spirals and bipolar polytrichous tufts of flagella
   c. No growth on artificial media

X and V Factors and Susceptibility Testing for Haemophilus

Isolation of Legionella

Other Fastidious Gram Negative Rods

ORGANISM	DESCRIPTION	DISEASE	NOTES
Bordetella pertussis	Gram Neg Coccobacillus; Pinpoint, "Mercury Droplet" Colonies	Pertussis *(Whooping Cough)*	Bordet-Gengou Media; NP Swab and Plate Directly
Haemophilus	Small, Non-Motile Gram Neg Rods • *H. ducreyi* - "School of Fish"	*H. influenzae* - Causes Influenza, Meningitis, and Epiglottitis *H. ducreyi* - Causes Genital Ulcers	Require X and V Factors
Legionella pneumophilia	Growth on BCYE	Legionnaires' Disease	No Growth Routine Media
Mycoplasma/Ureaplasma	Colony Appears as Inverted Fried Egg	*M. pneumoniae* - Causes Primary Atypical Pneumonia *(↑ Cold Agglutinin Titer)*	Dienes Stain NOT Gram Stain

Anaerobes

1. **Clues to anaerobic infection**
   a. **Foul odor to specimen**
   b. **Location in close proximity to a mucosal surface**
   c. **Animal or human bite**
   d. **Gas in specimen**
   e. **Previous therapy with aminoglycosides**
   f. **Black discoloration of blood containing exudates**
   g. **Presence of "sulfur granules"**
   h. **Unique morphology on gram stain**
   i. **Failure to grow organisms seen on smear aerobically**
   j. **Growth in anaerobic zone or bubbles in fluid media**

2. **Specimen collection and transport**
   a. **Site containing a resident flora (*oral, GI, GU*) not appropriate for anaerobic culture**
   b. **Best to aspirate with syringe and needle and place in a transport vial or tube under reduced conditions (*swab samples not as good*)**

3. **Culture techniques**
   a. **"Classic" principle of anaerobic culture**
      ❖ ***Jar technique (Gas Pak jar)***
      ❖ ***Catalyst - palladium pellets***
      ❖ ***Envelope generates H_2 and CO_2 when water is added***
      ❖ ***Methylene blue or resazurin - indicators (blue and pink, respectively when oxidized; clear when reduced)***
   b. **Other methods**
      ❖ ***Anaerobic bags - clear bag with gas generating ampules; plates can be read without opening bag***
      ❖ ***Roll tube technique***
         ☞ **PRAS (*pre-reduced anaerobically sterilized media*) inoculated under constant flow of O_2 - free gas**
      ❖ ***Anaerobic chamber***
         ☞ **Plates put in chamber through a pass box that is reduced**
         ☞ **Incubator in chamber; also contains palladium catalyst**

EXAMINATION OF PRIMARY PLATE

1. Pitting - *Bacteroides ureolyticus (could be Eikenella)*
2. Large colonies with double zone of hemolysis - *Clostridium perfringens*; set up egg yolk agar for Naegler test
3. Bread crumb or speckled colonies; gram negative slender fusiforms - *Fusobacterium nucleatum*
4. Molar tooth colonies of Gram positive branching rods - *Actinomyces*
5. BBE - dark colonies, > 1 mm - *Bacteroides fragilis*
6. KVLB - look for pigment and examine under UV light
   a. Perform aerotolerance test on colonies by subculturing each colony type to an anaerobic blood plate and a chocolate plate
   b. Incubate chocolate at 36°C under 5-10% CO_2 and the anaerobe BAP at 36°C in a gas jar or anaerobe chamber
   c. If growth on both, the organism is facultative; if growth only on anaerobic blood, the organism is an anaerobe

Differentiating Gram Negative Anaerobes

	Vancomycin *(5 μg)*	Kanamycin *(1 mg)*	Colistin *(10 μg)*	Indole	Lipase	Esculin	H_2S	Notes
Bacteroides fragilis	R	R	R	V	—	+	—	Catalase +, black colonies on BBE
Pigmented Gram Negative Rods	S	R	V	+	V	—	—	May fluoresce brick-red, may produce black pigment
Bacteroides ureolyticus	R	S	S	—	—	—	—	Pits agar; urease +
Fusobacterium nucleatum	R	S	S	+	—	—	—	Thin, fusiform rods, speckled colonies
Fusobacterium necrophorum	R	S	S	+	+	—	+	Rods variable in length and width
Fusobacterium mortiferum	R	S	S	V	—	+	+	Highly pleomorphic rods

R = resistant S = sensitive V = variable

Differentiating Gram Positive Anaerobes

ORGANISM	CHARACTERISTICS
Clostridium difficile	Pseudomembranous Colitis (PMC); Usually ID with immunodiagnostic tests; Emerging resistant strains; CCFA Agar ("Horse Stable" Odor); Spore Former
Clostridium perfringens	Double Zone of Hemolysis; Lecithinase +; Gas Gangrene; Spores Seldom Observed
Clostridium tetani	Terminal Spores ("Racquet-Shaped"); Tetanus
Actinomyces israelii	"Molar Tooth" Colony; Branching Gram + Rods ("Lumpy Jaw"); Sulphur Granules
P. anaerobius	Sensitive to SPS

Organism associated with PMC

Organism Associated with Lumpy Jaw and "Sulfur" Granules

Spirochetes

TREPONEMA PALLIDUM

1. Stain with silver impregnation
2. Darkfield - slow motility and flexion
3. No growth on artificial media
4. Sensitive to penicillin, tetracycline and erythromycin

5. Detected through serological tests (*see Serology/Immunology for details*)

6. Syphilis
   a. Primary lesion
      - ❖ *2-10 weeks after infection, chancre appears*
      - ❖ *Heals without treatment in 3-8 weeks; may perform darkfield or direct FA on fluid*

   b. Secondary lesion
      - ❖ *Dissemination - skin rash*

   c. Latent stage
      - ❖ *2-20 yrs later*
      - ❖ *Affects skin, bone, joints, CNS*

LEPTOSPIRA

1. Spirals with hooked ends

2. Animal pathogen passed to humans via water contaminated with animal urine *(ex. sewer workers, farmers, vets)*

3. Positive darkfield or direct FA

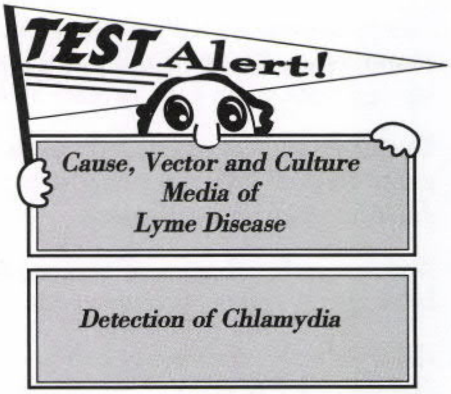
4. Can grow in Fletcher's semi-solid media
   a. Incubate 6 wks at 30°C in the dark
   b. Perform darkfield from several centimeters into media

Media for Culturing Leptospira

BORRELIA

1. *B. recurrentis*
   a. "Relapsing fever" from ticks or lice
   b. Looser coils; best seen with Giemsa or Wright's stain of blood smear
   c. Mutates during disease; relapse due to inability to recognize new antigen
   d. May exhibit cross reaction with Proteus OX K on febrile agglutinations with titer up to 1:80

2. *B. burgdorferi*
   a. Lyme disease
   b. Transmitted by *Ixodes* ticks (*deer or mouse tick)*
   c. Northeastern and N. Midwest US
   d. Chronic migratory erythematous rash, fever, muscle and joint pain; later meningioencephalitis, myocarditis and arthritis
   e. Culture in Kelly medium at 33°C - darkfield weekly for 1 month
   f. Serological diagnosis faster



Cause, Vector and Culture Media of Lyme Disease

Detection of Chlamydia

Chlamydiales

1. Obligate intracellular parasites

2. Gram negative cell wall, with no peptidoglycan; possess ribosomes for protein synthesis

3. Dependent on host for ATP

4. Laboratory diagnosis
   a. Giemsa stain (*purple inclusion bodies*) or iodine stain

5. Growth in yolk sac of chick embryo or tissue culture (*McCoy cell*)

6. *Chlamydia trachomatis*
   a. Genital tract infections (*sexually transmitted disease)*
      - ❖ *Non-gonococcal urethritis and epididymitis in males*
      - ❖ *Cervicitis and salpingitis (PID) in females*
      - ❖ *Can be passed to newborn as conjunctivitis or pneumonia*

   b. Eye infections
      - ❖ *Trachoma - leading cause of blindness in underdeveloped countries*
      - ❖ *Inclusion conjunctivitis*

   c. Most ID by probe technology

7. *Chlamydophila psittaci*
   a. Psittacosis (*parrot fever*) - occupational hazard for pet bird handlers and poultry workers
   b. Acute lower respiratory infection

8. *Chlamydophila pneumoniae*
   a. Grown in HELA cells
   b. Respiratory pathogen
   c. Linked to cardiovascular disease

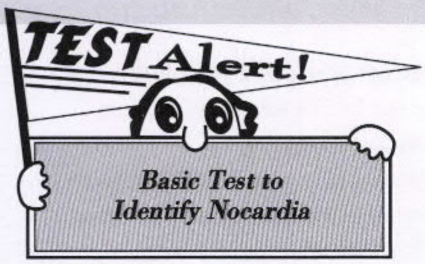
Rickettsiae

1. Small gram negative coccobacilli
2. Obligate intracellular parasites
3. Spread by arthropod (insect) vectors
4. Seen better with Giemsa
5. Arthropod bite - causes fever, headache, rash *(Q fever - no rash and organism survives outside host)*
6. Weil-Felix test
   a. *Proteus* OX-19, OX-2 and OX-K used as antigens to detect Rickettsial antibody
   b. 4-fold rise in titer or 1:160 titer

Most Common Rickettsiae

ORGANISM	VECTOR	PROTEUS			NOTES
		OX 19	OX 2	OX K	
R. akari (Rickettsial Pox)	House Mites	–	–	–	
Coxiella burnetti (Q Fever)	Inhaled	–	–	–	Confirm with CF Test
R. prowazekii (Typhus Fever)	Louse	+	Variable	–	
R. rickettsiae (Rocky Mt. Spotted Fever)	Tick	+	+	–	Characteristic Rash on Palms of Hand and Soles of Feet
R. typhi (Murine Typhus)	Rat Flea	+	+	–	

Acid Fast Bacilli


TEST Alert!
Basic Test to Identify Nocardia

Mycobacteria

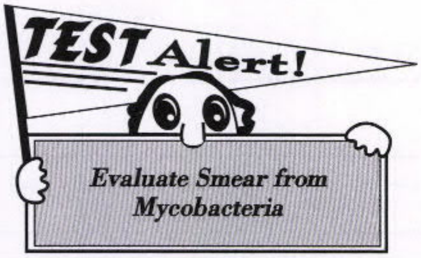
1. Morphology - slim gram variable rods; don't gram stain well due to high lipid content in wall
2. Acid fast stain
   a. Ziehl-Neilsen - "hot" stain
   b. Kinyoun - "cold" stain
3. Auramine-Rhodamine - fluorescent stain
4. All stains based on presence of mycolic acid (*lipid-wax*) in cell wall
5. Any number seen on a smear is significant
6. Growth requirements
   a. Lowenstein-Jensen; 60% egg in nutrient base; malachite green; solidified into slants after inspissation (heat to 85°C until protein coagulates)
   b. Tween 80 (*oleic acid*) - aids in dispersing colonies in liquid media
   c. ↑CO_2 (*especially in first 24 hrs*)
   d. Most grow at 36°C; some require 30°C
   e. Takes 3-6 weeks for growth
   f. Automated liquid culture systems for rapid growth & susceptibility testing
7. Identification by nucleic acid probe

SPECIMEN COLLECTION

1. Sputum *(first morning; on 3 consecutive mornings)*, bronchial & gastric washings, urines and tissue
2. Collect aseptically and place in sterile, tightly capped container
3. May be refrigerated overnight (*neutralize gastrics and urines if holding overnight*)

Branching Gram Positive Rods

ORGANISM	ATMOSPHERE	CASEIN	TYROSINE	XANTHINE	ACID FAST
Actinomyces israeli	Anaerobic	N.A.	N.A.	N.A.	N.A.
Nocardia asteroides	Aerobic	—	—	—	Partially
Nocardia brasiliensis	Aerobic	+	+	—	Partially



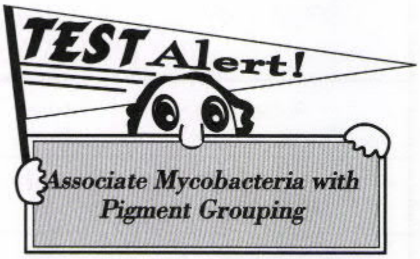
Evaluate Smear from Mycobacteria

Collection and Handling of Mycobacterial Specimens

SPECIMEN DECONTAMINATION

1. N-acetyl-L-cysteine (*NALC*) - mucolytic; NaOH decontaminates; time dependent
2. Stain and report slides within 24 hrs of processing
3. Centrifuge decontaminated specimen for 20 min at 3000 rpm prior to making smears and inoculating media (*use sediment)*

(See Mycobacteria chart on following page.)



Associate Mycobacteria with Pigment Grouping

I.D. and Rapid Reporting of M. tuberculosis

REMEMBER!

Mycobacterium tuberculosis

- ***Infects 1/3 of world's population***
- ***Is spread by respiratory droplets***
- ***Is resistant to drying***
- ***Is resistant to many disinfectants***
- ***Is best known for respiratory disease***
- ***Has "cauliflower" colonies on LJ***
- ***Requires long-term treatment***
- ***Has MDR variants***
- ***Can be grown in automated systems***
- ***Can be identified by nucleic acid probes***
- ***Use skin test for screening in U.S.***

Mycobacterium avium complex

- ***Are environmental organisms***
- ***May cause pulmonary disease***
- ***May cause disseminated disease***
- ***May infect immunocomprised patients***
- ***Non-pigmented colonies on LJ***
- ***Can be identified by nucleic acid probes***

Mycobacterium leprae

- ***Causes leprosy (Hansen disease)***
- ***Infects skin, mucous membranes, nerves***
- ***Causes a progressive disease that is treatable***
- ***Grows best in armadillo footpads***

Differentiating Mycobacteria

	Growth < 7 days on traditional media	Niacin	Nitrate Reduction	Tween 80 Hydrolysis	Tellurite *(3 days)*	Growth on MacConkey	Notes
M. tuberculosis	–	+	+	> 5 days	–	–	Rough & buff, serpentine cording on culture
M. bovis	–	–	–	> 5 days	–	–	Rough & buff colony, serpentine cording; susceptible to TCH
M. kansasii	–	–	+	< 5 days	–	–	Photochromogen
M. marinum	–	v	–	< 5 days	–	–	Photochromogen *(30° C optimum temperature)*
M. scrofulaceum	–	–	–	–	–	–	Scotochromogen
M. gordonae	–	–	–	5-10 days	–	–	Scotochromogen
M. avium	–	–	–	–	+	–	Non-photochromogen
M. ulcerans	–	–	–	–	–	–	Non-photochromogen *(32° C optimum temperature)*
M. fortuitum	+	–	+	–	v	+	Rapid grower
M. chelonei	+	v	–	–	v	+	Rapid grower

v = variable
Shaded areas = Key reactions differentiating similar genera

Photochromogen - *produces pigment in light*
Scotochromogen - *produces pigment in dark and light*
Non-photochromogen - *produces no pigment*
Rapid grower - *growth in < 7 days*

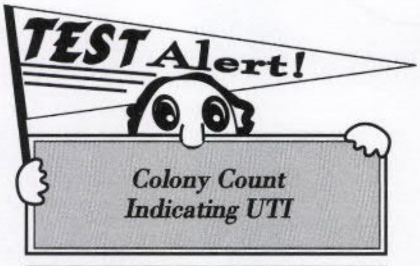
Specimen Source and Potential Pathogens

BODY SITES/SOURCE	“NORMAL” FLORA	EXPECTED PATHOGEN	NOTES
Throat, oropharynx	Alpha *Strep* *Staph* sp. *Neisseria* sp. Gram + Rods Anaerobes	*Strep* Group A	
		Corynebacterium diphtheriae	Pseudomembranes when toxin produced
		Bordetella pertussis	Whooping cough; confirm with fluorescent antibody test
		Haemophilus influenzae	Acute epiglotitis in children *(Do not attempt throat culture; blood culture best.)*
Eye	Same as above except in lesser numbers	*Chlamydia*	Newborns
		Neisseria gonorrhoeae	Newborns
Ear	“	*Pseudomonas aeruginosa*	Swimmer's ear
		H. influenzae	Otitis media
		Streptococcus pneumoniae	Otitis media
Lower Respiratory Tract *(sputum)*		*H. influenzae*	Early morning sputum specimen best; should have less than 10 - 15 squamous epithelial cells per LPF *(represents oral flora contamination)*
		S. aureus	“
		Streptococcus pneumoniae	“
		Klebsiella pneumoniae	“
		Mycoplasma pneumoniae	“
		Mycobacterium tuberculosis	“
		Legionella	“
		Fungi	“
Transtrachial Aspirate, Lung Tissue	Should be sterile	Anaerobes	
Bronchial Washing	Resident oral flora		No anaerobic set-up; AFB and mycology set-up
Gastric Specimens		*Helicobacter pylori*	Rapid urease test
Colon	Profuse flora	*Shigella*	Selective and differential media on stools *(MAC, EMB, HE, XLD, selenite, or GN broth)*; subculture to selective media after 6 - 12 hours
		Salmonella	“
		Campylobacter jejuni	Microaerophilic at 42°C
		Yersinia entercolitica	MAC after 48 hours at room temperature
		Vibrio cholerae	TCBS
		Toxigenic *E. coli*	Other tests better than culture
		Clostridium difficile	EIA best

BODY SITES/SOURCE	"NORMAL" FLORA	EXPECTED PATHOGEN	NOTES
Urinary Tract	Normally sterile *(May be contaminated with fecal flora)*	*E. coli*	Midstream catch with proper skin preparation; >100,000 organisms per ml for infection *(work up smaller number if pure culture and white cells present)*
		Other gram neg rods	"
		E. faecalis	"
		Staphylococcus sp.	"
Genital Tract		*Neisseria gonorrhoeae*	Male - purulent discharge, do gram stain; Female - gram stain not sensitive or specific enough, do culture for GC *(Thayer-Martin: selective media for GC)*
		Chlamydia trachomatis	Serological tests more accurate and faster
		Strep Group B	Significant in pregnant women
		Herpes simplex	
		Trichomonas vaginalis	Parasite, wet mount
		Treponema pallidum	Darkfield
		Gardnerella vaginalis	Implicated in vaginosis; look for "clue cells"
CSF	Normally sterile	*H. influenzae*	Children under 5
		Neisseria meningitidis	
		E. coli	Neonates
		Strep Group B	Neonates
		Cryptococcus	Immunocompromised patients
		Listeria	Immunocompromised patients
Deep Wounds/Abcesses		Anaerobes and Aerobes; depends on site	Bypass normal flora in collection; needle aspirate better than swab
Superficial Wounds *(pustules, dermatitis, rashes)*		*S. aureus*	
		Strep Group A	
		Pseudomonas aeruginosa	
Blood	Normally sterile		In immunocompromised and prosthetic heart device patients, any organism is considered pathogenic

Gram Positve Cocci Found in Sputum

Organism Associated with Peptic Ulcers



Colony Count Indicating UTI

Specimen Collection and Handling of NP Swab

Virology

VIRAL STRUCTURE

1. RNA or DNA - not both
2. Does NOT contain structural elements required for protein synthesis
3. Replicates in host cells

SPECIMEN COLLECTION AND HANDLING

1. Pre- and post-convalescent sera - ship on dry ice
2. Specimen for viral culture - similar to transport media for bacteria but contains nutrients (*fetal calf serum or albumin*) and antibiotics

LABORATORY METHODS

1. EIA - presence of viral antibody or antigen *(ex. HbsAg and anti-HBsAb)*
2. Viral culture
3. Electron microscopy
4. Molecular techniques

SPECIAL PROCEDURES

1. DNA probes
   a. Molecular cloning of a specific nucleic acid sequence
   b. If unknown "matches" clone, the viral identity is confirmed
2. Polymerase chain reaction (*PCR*)
   a. Method in which nucleic acid sequences can be amplified in vitro
   b. Carried out in cycles, each cycle doubling the amount of desired nucleic acid product
   c. See Molecular Diagnostics section for blot and other technologies.

Collection and Handling of Viral Specimens

DNA Probe Technology

PCR Technology

REMEMBER!

Viruses

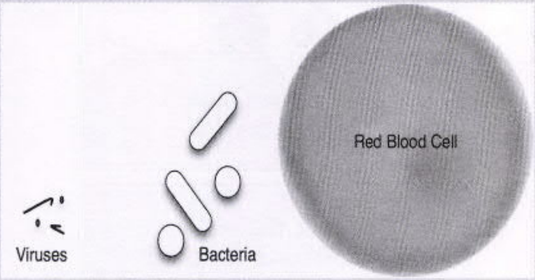
* Are very small

* Are not cells

* Reproduce inside host cells

* Consist of nucleic acid and a protein coat

* Infect specific cell types



RNA Viruses

VIRUS	DISEASE	NOTES
Flavivirus	Yellow Fever; Dengue; St. Louis Encephalitis	Mosquito - Vector
Hantavirus	Pulmonary Syndrome; Hemorrhagic Fever	Rodent-Borne
Hepatitis A Virus *(HAV)*	Hepatitis A	Associated with Shellfish; One of Most Stable Viruses Infecting Humans
Hepatitis C Virus *(HCV)*	Hepatitis C	Formerly Non-A, Non-B Hepatitis
Influenzavirus	Influenza	
Morbillivirus	Measles	More Serious in Adults than Children
Mumps Virus	Mumps	
Parainfluenza Virus	Parainfluenza	
Poliovirus	Poliomyelitis; Aseptic Meningitis	Occurs Naturally Only in Humans
Respiratory Syncytial Virus *(RSV)*	Serious Respiratory Infection in Young Children	Giant Multinucleated Cells Due to Fusion of Infected Cells
Rhabdovirus	Rabies	Negri Bodies in Brain Tissue of Infected Animals; Rod or Bullet-Shaped; Wildlife - Reservoir
Rhinovirus	"Common" Cold	
Rotavirus	Acute Infectious Infantile Diarrhea	Can Cause Death in Infants
Rubivirus	Rubella	Vaccine Available; Contraindicated in Pregnancy; Spread by Respiratory Secretions; Serious Congenital Abnormalities
RETROVIRUS		
Human Immunodeficiency Virus *(HIV I/II)*	AIDS	EIA Techniques; Confirmed by Western Blot
Human T-Cell Leukemia Virus *(HTLV I)*	T-Cell Leukemia; Tropical Spastic Paraparesis *(TSP)*	EIA Techniques; Confirmed by Western Blot
Human T-Cell Leukemia Virus *(HTLV II)*	Hairy-Cell Leukemia	EIA Techniques; Confirmed by Western Blot

TEST Alert!

RNA Viruses and Associated Diseases

RSV-Associated Disease and I.D.

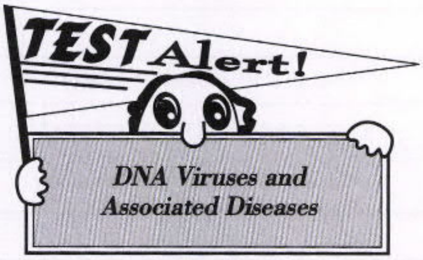
TEST Alert!

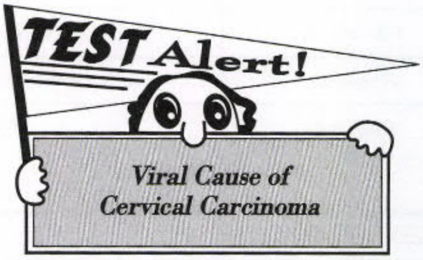
Evaluate Lab Results to Determine Viral Disease

Virus Associated with Negri Bodies

DNA Viruses

VIRUS	DISEASE	NOTES
Adenovirus	Respiratory Infections	
Cytomegalovirus *(CMV)*	Mental Retardation *(most common viral cause)*; Other Problems in Immunosuppressed Depending on Site of Infection	Most Common Congenital Infection
Epstein-Barr Virus *(EBV)*	Infectious Mononucleosis; Chronic Fatigue Syndrome; Associated with Burkitts Lymphoma	Heterophile Antibody
Hepatitis B Virus *(HBV)*	Hepatitis B	ELISA Techniques; Vaccine Available
Herpesvirus *H. simplex* I *H. simplex* II	Oral Infections Genital Infections *(STD)*	
Human papillomavirus (HPV)	Genital Warts; Cutaneous Warts	Some Serotypes Associated with Cervical Carcinoma; Vaccine available
Poxvirus	Smallpox	Supposedly Eradicated; Occasional Outbreaks in Labs Where Virus Cultures Are Stored
Varicella-Zoster	Chicken Pox *(children)*; Shingles *(adults)*	Diagnosed by Clinical Picture


TEST Alert!
DNA Viruses and Associated Diseases


TEST Alert!
Viral Cause of Cervical Carcinoma

REMEMBER!

The most important thing in science is not so much to obtain new facts as to discover new ways of thinking about them.

— Sir William Bragg

MICROBIOLOGY SAMPLE QUESTIONS

1. A "rice water stool" is characteristic of patients infected with
   - A. *Clostridium botulinum.*
   - B. *Salmonella typhi.*
   - C. *Shigella dysenteriae.*
   - D. *Vibrio cholerae.*

2. A sample of material from bluish purulent head lesions is submitted for analysis. A gram negative, motile, non-sporeforming oxidase positive rod was isolated. This organism is most likely
   - A. *Proteus mirabilis.*
   - B. *Proteus vulgaris.*
   - C. *Pseudomonas aeruginosa.*
   - D. *Stenotrophomonas maltophilia.*

3. A gram stained smear from a genital soft chancre demonstrated small gram negative rods arranged in tangled chains. You would suspect the cause of the chancroid to be
   - A. *Hemophilus ducreyi.*
   - B. *Herpes simplex II.*
   - C. *Neisseria gonorrhoeae.*
   - D. *Treponema pallidum.*

4. Organisms of this genus are gram negative, motile rods. A few are chromogenic and produce a red non-water soluble pigment. Some have been implicated in septicemia, pulmonary and urinary tract infections. One member of this genus is
   - A. *Pseudomonas aeruginosa.*
   - B. *Sarcina lutea.*
   - C. *Serratia marcescens.*
   - D. *Staphylococcus aureus.*

5. Which characteristic is most useful in differentiating *Citrobacter* and *Salmonella*?
   - A. H_2S production
   - B. Indole production
   - C. Lysine decarboxylase
   - D. Urease production

6. A gram stain from a sputum specimen demonstrates many gram positive cocci in chains and pairs. Numerous small alpha streptococci are observed on the primary blood agar plate. To determine if these organisms are *Streptococcus pneumoniae* which of the following tests should be performed?
   - A. Bacitracin susceptibility
   - B. Catalase
   - C. Esculin hydrolysis
   - D. Optochin susceptibility

7. Loeffler's medium is used as a primary isolation medium for
   - A. *Bordetella pertussis.*
   - B. *Corynebacterium diphtheriae.*
   - C. *Mycobacterium tuberculosis.*
   - D. *Streptococcus pyogenes.*

8. Autoclave sterilization of lab media requires which of the following pressure, temperature and time parameters?
   - A. 15 lbs pressure, 115°C for 10 min
   - B. 15 lbs pressure, 121°C for 15 min
   - C. 20 lbs pressure, 100°C for 15 min
   - D. 20 lbs pressure, 110°C for 10 min

9. Clinical diagnosis of rabies in infected animals is dependent upon brain tissue observation of
   - A. Metachromatic granules.
   - B. Multinucleated cells.
   - C. Negri bodies.
   - D. Viral capsids.

10. Which of the following results is typical of *Campylobacter jejuni*?
    - A. Catalase negative
    - B. Non-motile
    - C. Optimal growth at 42°C
    - D. Oxidase negative

11. The use of penicillin/aminoglycoside to treat endocarditis due to *Streptococci* Group D represents a
    - A. Broad spectrum susceptibility.
    - B. Multi-drug resistance.
    - C. "Shotgun" approach.
    - D. Synergistic relationship.

12. Characteristics indicating an appropriate sputum collection would be microscopic findings of
    - A. > 25 epithelial cells, > 25 white cells.
    - B. > 25 epithelial cells, < 25 white cells.
    - C. 10-25 epithelial cells, 10-25 white cells.
    - D. < 10 epithelial cells, 10-25 white cells.

13. A clean catch urine specimen from a female with a suspected UTI showed gram positive cocci that were catalase positive, coagulase negative and Staph latex negative. On the Microscan panel, growth in the novobiocin well was noted. The most likely organism is
    - A. *Staphylococcus aureus.*
    - B. *Staphylococcus epidermidis.*
    - C. *Staphylococcus saprophyticus.*
    - D. *Enterococcus fecalis.*

14. The treatment of choice for methicillin resistant *Staphylococcus* is
    - A. Cephalothin.
    - B. Nafcillin.
    - C. Oxacillin.
    - D. Vancomycin.

15. The most likely cause of subacute bacterial endocarditis is
    - A. *Staphylococcus aureus.*
    - B. *Staphylococcus epidermidis.*
    - C. *Streptococcus Group A.*
    - D. *Streptococcus viridans.*

16. The organism known for its "stormy fermentation" and double zone of beta hemolysis under anaerobic conditions causes
    - A. Botulism.
    - B. Gas gangrene.
    - C. Pseudomembraneous colitis.
    - D. Tetanus.

17. The bacteremic Waterhouse-Friderichsen syndrome is associated with
    - A. *Corynebacterium jeikeium.*
    - B. *Listeria monocytogenes.*
    - C. *Mycoplasma pneumoniae.*
    - D. *Neisseria meningitidis.*

18. The specimen of choice in a case of suspected epiglottitis is collected from the
    - A. Blood.
    - B. Spinal fluid.
    - C. Sputum.
    - D. Throat.

19. A specimen from a female complaining of vaginitis emitted a "fishy" odor when mixed with 10% KOH. A wet prep showed some white cells and epithelial cells covered with small gram variable rods. The most likely organism is
    - A. *Chlamydia trachomatis.*
    - B. *Gardnerella vaginalis.*
    - C. *Neisseria gonorrhoeae.*
    - D. *Treponema pallidum.*

20. A photochromogenic mycobacterium isolated at 30 C is most likely
    - A. *M. gordonae.*
    - B. *M. marinum.*
    - C. *M. ulcerans.*
    - D. *M. xenopi.*

21. A scotochromogenic mycobacterium showing hydrolysis of Tween 80 in 7 days is probably
    - A. *M. avium.*
    - B. *M. fortuitum.*
    - C. *M. gordonae.*
    - D. *M. marinum.*

22. A gram negative rod, isolated from the urine of a female with recurrent UTI, was oxidase negative, urease positive showing A/A with H2S on TSI and red/black on LIA is most likely
    - A. *Escherichia coli.*
    - B. *Klebsiella pneumoniae.*
    - C. *Proteus mirabilis.*
    - D. *Pseudomonas aeruginosa.*

23. An organism recovered from a diarrheal stool was K/A with no gas or H2S on TSI, lysine negative, oxidase negative, urease negative and citrate negative. The most likely organism is
    - A. *Aeromonas hydrophila.*
    - B. *Escherichia coli.*
    - C. *Proteus vulgaris.*
    - D. *Shigella sonnei.*

24. A cause of acute infectious infantile diarrhea is
    - A. Hantavirus.
    - B. HIV.
    - C. Rhabdovirus.
    - D. Rotovirus.

25. Specimens for viral culture should be transported in
    - A. Anaerobic containers.
    - B. Bovine albumin (22%).
    - C. Nutrient medium with antibiotics.
    - D. Sheep blood (5-10%).

ANSWERS AND RATIONALE

1. D

Though options B and C may cause diarrhea, only *V. cholera* causes the characteristic "rice water stool". Option A may cause infant botulism which is characterized by 2-3 days of constipation followed by flaccid paralysis.

2. C

Pseudomonas aeruginosa is the only oxidase positive organism listed.

3. A

Option B is a virus not visible on a gram stain. Option C are gram negative diplococci. Option D is a spirochete that causes a hard chancre.

4. C

The only organism listed which produces a red pigment is *Serratia.* Options B and D are cocci.

5. C

The classic biochemical reaction which separates these two genera is lysine. The other biochemical reactions can be variable.

6. D

Optochin susceptibility separates *Streptococcus pneumoniae* from the other Streptococci. Option A is a characteristic of *Streptococcus pyogenes.* Option B separates *Staphylococcus* (*catalase positive*) from *Streptococcus (catalase negative*). Group D *Streptococcus* are positive with option C (*esculin hydrolysis).*

7. B

Microscopic morphology is best demonstrated on Loeffler's medium though cystine-tellurite agar is also used (*C. diphtheriae colonies demonstrate a grey-to-black color on this media*). Option A is seen on Bordet-Gengou media. Option C is associated with Lowenstein Jensen media. Option D grows well on blood agar.

8. B

Autoclave sterilization requires 15 psi, at 121°C for 15 min.

9. C

Demonstration of Negri bodies (*cytoplasmic inclusion bodies*) in brain tissue is the hallmark of rabies diagnosis. Option A is not seen in viruses. Option B is associated with measles virus. Option D is seen in electron micrographs of many viruses and bacteria.

10. C

Campylobacter jejuni is oxidase positive, motile, catalase positive and grows optimally at 42 C.

11. D

These drugs in combination enhance bactericidal activity.

12. D

Greater than 10 epithelial cells indicates the specimen is heavily contaminated with oral flora.

13. C

S. saprophyticus is resistant to novobiocin and can cause urinary tract infections.

14. D

Vancomycin is the drug of choice for methicillin resistant *Staphylococci.*

15. D

Streptococcus viridans is most commonly associated with subacute bacterial endocarditis.

16. B

Clostridium perfringens is the cause of gas gangrene and is noted for its stormy fermentation and double zone of beta hemolysis. Option A is caused by C. botulinum and is diagnosed by demonstration of botulism toxin. Option C is caused by C. difficile which grows on CCFA agar. Option D is caused by C. tetani and is identified by its "racquet' or "drumstick" shaped terminal endospores.

17. D

Overwhelming DIC (due to large amounts of endotoxin) with shock and destruction of adrenal glands is caused by N. meningitidis.

18. A

Options B and C are not related to the diagnosis. Collecting a throat culture (*option D)* could cause the airway to close.

19. B

***Gardnerella* is associated with the haracteristic "fishy" odor when vaginal discharge is mixed with KOH. Option A is diagnosed by EIA, DNA probes or FA. Option C are gram negative cocci. Option D is a spirochete seen with darkfield microscopy.**

20. B

Option A is a scotochromagen. Option C grows at 30°C but is a non-photochromagen. Option D is a scotochromagen and grows best at 37°C.

21. C

Option A is a non-photochromogen and does not show hydrolysis of Tween 30. Option B is a rapid grower and does not show hydrolysis of Tween 80. Option D is a photochromogen which hydrolysis Tween 80 in less than 5 days.

22. C

Options A and B are H_2S negative and deaminase negative. Option D is K/K on TSI and oxidase positive

23. D

Option A is oxidase positive. Option B is A/A on TSI and indole positive. Option C is H_2S positive and urease positive

24. D

Option A causes hemmorrhagic fever. Option B causes AIDS. Option C causes rabies.

25. C

Media for transporting specimens for viral culture are similar to bacterial transport media but must contain additional nutrients such as albumin or fetal calf serum and antibiotics (*to prevent bacterial growth).*

MYCOLOGY

by Mary Lux and Michele Zitzmann*

Characteristics of Fungi

1. Reproduction
   a. Sexual - fusion of 2 haploid nuclei; spores - teleomorph
   b. Asexual - mitotic division of haploid nucleus and budding production of conidia - anamorph

2. Growth and morphology
   a. Diverse; from bacteria-like yeast to mushrooms
   b. Hyphae
      ❖ *Tube-like structures with thick parallel walls*
         ☞ Septate - has cross walls
         ☞ Aseptate (*coenocytic*) - has rare cross walls
      ❖ *Several types: racquet, favic chandeliers, pectinate, nodular, spiral*
      ❖ *Mycelium is a mat of hyphae*
         ☞ Vegetative growth into medium
         ☞ Aerial growth above the medium
   c. Pseudohyphae are elongated budding yeast cells (*blastoconidia*) with constrictions between cells (*buds*)
   d. Fruiting bodies
      ❖ *Sexual (perfect, teleomorphic)*
         ☞ *True sporulation*
         ☞ Fusion of haploid nuclei
         ☞ Homosexual - zygospore
         ☞ Heterosexual - oospore
         ☞ Spore sacs (*asci*)
            ✯ *Cleistothecium (round, closed)*
            ✯ *Perithecium (flask-shaped, open)*
            ✯ *Apothecium (saucer-shaped, open)*
      ❖ *Asexual (imperfect, anamorphic)*
         ☞ From specialized supportive hyphae
            ✯ *Chlamydoconidia - round, thick-walled structures located terminal, intercalary, sessile*
            ✯ *Arthroconidia - hyphal fragmentation at cross walls*
         ☞ Aerial structures
            ✯ *Conidiophore, vesicle, phialiade, conidium*
            ✯ *Sporangiophore, columella, sporangium, spore*
   e. Mycelial structures for Zygomycetes - stolon, rhizoid

CLASSIFICATION

1. *Zygomycota* - ribbon-like aseptate hyphae; sexual and asexual

2. *Ascomycota* - septate; sexual and asexual; produce asci

3. *Basidiomycota* - septate; sexual; mushrooms; club fungi

Laboratory Methods

1. 10% KOH wet prep
   a. Clears debris and breaks down keratin from nails and hair
   b. Use with specimen

2. Lactophenol cotton blue
   a. Stains and kills organism
   b. Use with culture material

3. India ink - capsule of *Cryptococcus neoformans*

4. Calcofluor white - fluorescent

5. Primary growth agars
   a. Sabouraud's Dextrose agar (*SAB*) - glucose, peptone, pH 5.6
   b. Mycosel - similar to SAB but contains cycloheximide, BHI with 5% SRBC's, gentamicin and chloramphenicol
      ❖ *Inhibits some Candida and Cryptococcus; also Aspergillus fumigatus and Pseudallescheria boydii*

6. Specialty growth media
   a. Bird-seed agar - *Cryptococcus neoformans (brown colonies)*
   b. Corn meal agar - *Candida albicans* (*chlamydoconidia*)
   c. Rice infusion oxgall Tween 80 (*RIOT*)- chlamydoconidia

Specimen Collection and Handling

1. Optimum temperature - 25-30°C

2. Hold 6 weeks

3. Use screw-cap tubes or tape plates to avoid accidental opening and drying

4. Work under a biologic safety hood

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*Original contribution by Susan M. Marler (deceased)

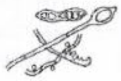
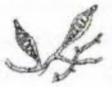
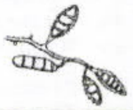
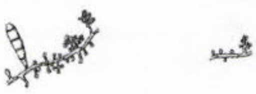
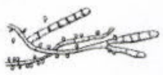
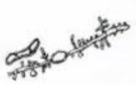
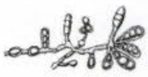
Processing Samples for Fungal I.D.

DERMATOPHYTES

1. Keratinolytic
2. Tinea - ringworm
3. Septate hyphae; micro- and macroconidia
4. Cause tinea capitis
   a. Endothrix (*inside shaft*) - temporary hair loss
   b. Ectothrix *(outside shaft)* - permanent hair loss
5. KOH prep of scales from advancing margin of lesion, hair or nails
6. Wood's lamp - some dermatophytes fluoresce with UV light (*Microsporum in hair*)
7. Treatment - miconazole, clotrimazole, griseofulvin

SUPERFICIAL MYCOSES (HAIR, SKIN, NAILS)

Dermatophytes

Organism	Characteristics	Notes	Illustrations
Microsporum	Microconidia - Small Club-Shaped Macroconidia- Many, Rough, Spindle-Shaped (except M. audouinii)	Tinea (Mostly in Children); Hair and Skin; Hair Fluoresces	
M. audouinii		Rare Distorted Macroconidia; Terminal Chlamydoconidia	
M. canis		Thick-Walled Macro-conidia; Knobby End	
M. gypseum		Thin-Walled Macroconidia	
Trichophyton	Microconidia - Many Macroconidia - Few; Thin, Smooth Walls	Mostly in Adults; Hair Skin and Nails; NO Fluorescing Hairs	
T. mentagrophytes		Urease Pos; Rose-Brown Reverse	
T. rubrum		Urease Neg; Red Reverse	
T. tonsurans		Black Dot Ringwom; Balloon Forms; Yellow-Red Reverse	
Epidermophyton	Microconidia - None Macroconidia - 2-4 Cells in Clusters or Singles, Smooth Walls, Club-shaped	Skin and Nails; Especially Feet, Hands and Groin	
E. floccosum			

MICROSPORUM

1. Affects hair and skin
2. Mostly in children
3. Macroconidia - large, spindle shaped, rough with 4-15 septa
4. Microconidia - small, club shaped

M. audouinii

1. Epidemic tinea capitis
2. Hyphae usually sterile
3. Terminal chlamydoconidia
4. 10-21 days for growth
5. Reddish - brown color on reverse side of colony
6. Hairs fluoresce yellow-green

M. canis

1. Causes dog and cat ringworm which is passed to humans
2. Macroconidia - abundant with 4-8 septa, knoblike, echinulate (*rough*) ends
3. Thick walls
4. Growth 4-5 days
5. Yellow color on reverse side of colony
6. Ectothrix

M. gypseum

1. Many macroconidia with 3-5 septa and echinulate surface (*rounded ends, not knobby)*
2. Thin walls
3. Orange-brown color on reverse side of colony
4. Ectothrix

TRICHOPHYTON

1. Affects skin, hair and nails
2. Primarily in adults
3. NO fluorescing hair
4. Few or no macroconidia - thin, smooth walls
5. Many microconidia

T. mentagrophytes

1. Microconidia - numerous, spiral and nodular bodies; white, cottony mycelium
2. Rose-brown color on reverse side of colony
3. Endothrix
4. Urease positive in 2-3 days

T. rubrum

1. Microconidia - tear shaped and dispersed along hyphae
2. Cherry red color on reverse side of colony
3. Urease negative
4. Ectothrix

T. tonsurans

1. Causes black dot ringworm *(hair breaks off*)
2. Endothrix
3. Yellow-red color on reverse side of colony
4. Microconidia - numerous, clavate varying in size (*balloon forms and "matchstick" forms*)
5. Chlamydoconidia in older cultures

T. schoenleinii

1. Causes favus (*severe tinea capitis*)
2. Endothrix
3. Slow growth, waxy colonies, favic chandeliers (*look like "deer antlers"*) and chlamydoconidia

T. violaceum

1. Affects scalp and body
2. Wrinkled, yeast-like purple colony
3. Hyphae and chlamydoconidia in chains

EPIDERMOPHYTON

E. floccosum

1. Skin and nails (*especially feet, hands and groin)*

2. Macroconidia
    a. Large, smooth, club-shaped
    b. Found in singles or clusters at end of hyphae
    c. 2-4 septa
3. No microconidia
4. Olive green or khaki color

Tinea versicolor vs. nigra

	Tinea versicolor	Tinea nigra
Cause	*Malassezia furfur*	*Phaeoannellomyces werneckii* (*Dematiaceous*)
Clinical Description	Brown, Scaly Areas on Trunk, Arms, Face	Brown Patches on Hands
Description	Hyphae & Yeast-Like Spore on Direct Prep (*"Spaghetti & Meatballs"*)	Black Yeast-Like Colonies; Mold-Like with Age; 1-2 Clavate Cells
Miscellaneous	Overlay SAB with Olive Oil Prior to Inoculation	

Black vs. White Piedra

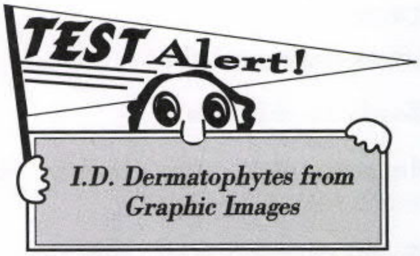
	Black Piedra	White Piedra
Cause	*Piedraia hortae* (*Dematiaceous*)	*Trichosporon beigelii*
Description	Black Nodules of Hyphae at Hair Shaft	White Nodules of Hyphae at Hair Shaft; White Mycelia; Arthroconidia and Blastoconidia

SYSTEMIC FUNGI

YEAST

Cryptococcus neoformans

1. Yeast; no pseudohyphae; brown colonies on bird seed agar
2. Mucoid colonies - capsule (*detected with India ink)*
3. Urease positive
4. Inhibited by cycloheximide
5. Found in pigeon and bird droppings
6. Meningitis (*found in spinal fluid*) and septicemia in immunocompromised hosts
7. Amphotericin B or flucytosine for treatment



I.D. Dermatophytes from Graphic Images

Micro/Macro Morphology of Dermatophytes

I.D. of Cryptococcus from India Ink Prep & Culture

Candida albicans

1. Yeast, pseudohyphae
2. Germ tube positive
3. Chlamydoconidia on cornmeal agar (CMAT)
4. Human flora

DIMORPHIC FUNGI

1. Yeast or tissue phase at 36°C; mold phase at 30°C
2. Treatment - Amphotericin B

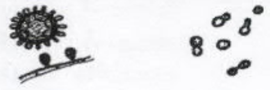
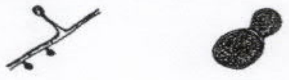
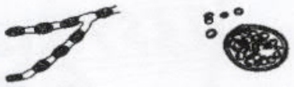
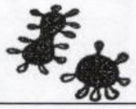
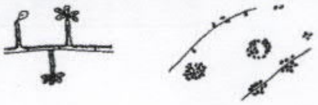
Histoplasma capsulatum

1. Small yeast forms in tissue
2. Mycelial form exhibits tuberculate macroconidia - diagnostic
3. Found in bird and bat droppings
4. Infects RES - bone marrow specimen of choice
5. Primary focus pulmonary
6. May be confused with *Sepedonium (show dimorphism by converting to yeast phase at 36°C on BHI with blood and without antibiotics)*

Blastomyces dermatitidis

1. Large yeast cells with broad based buds and double contoured wall; mold phase produces "lollipop" forms
2. Primary focus pulmonary; skin lesions common
3. Confused with *Scedosporium apiospermum* (*do conversion studies*)

Dimorphic Fungi

Organism	Characteristics	Notes	Illustrations
Histoplasma capsulatum	Yeast -Very Small Mycelial - Tuberculate Macroconidia	Bat and Bird Droppings; Ohio and Mississippi River Valley; Infects RES (Bone Marrow Specimen of Choice)	 Confused with Sepedonium
Blastomyces dermatitidis	Yeast - Broad-Based Bud; Double-Contoured Wall Mycelial - "Lollipop" Forms	Along Ohio, Mississippi Valley and Appalachia; May Cause Skin Lesions	 Confused with Scedosporium apiospermum
Coccidioides immitis	Yeast - Spherules Containing Endospores Mycelial - Alternately Staining Arthroconidia	Desert Southwest and Semiarid Regions	
Paracoccidioides brasiliensis	Yeast - Multiple Buds ("Mariner's Wheel") Mold - Similar to "Lollipop" Forms	"South American Blastomycosis"; Simulates TB; Cutaneous Lesions	
Sporothrix schenckii	Yeast - "Cigar" Bodies Mold - Delicate Hyphae with Ovoid Conidia Along Side ("Sleeve") or in Rosette Heads	Found in Dirt and On Plants ("Rose Gardener's" "Mycosis)	

Coccidioides immitis

1. Tissue form - large, round walled spherule containing endospores (*spherules vary in size)*
2. Mold phase - thick walled, alternate staining arthroconidia (*very infectious to lab personnel)*
3. Difficult to convert from mold to yeast phase in routine lab
4. Endemic in desert southwest and semiarid regions
5. Primary focus pulmonary

Paracoccidioides brasiliensis (South America blastomycosis)

1. Yeast form diagnostic - multiple blastoconidia budding from sides of large blastospore (*"Mariner's Wheel")*
2. Primary focus pulmonary; can simulate TB
3. May have cutaneous or mucocutaneous lesions

Subcutaneous Fungi

Sporothrix schenckii (Dimorphic)

1. Found in soil, plants, decaying matter
2. Traumatic inoculation through skin (*gardeners via rose thorns)*; usually on hand
3. Pyogenic and granulomatous inflammatory reaction
4. Yeast phase - ovoid, "cigar" bodies
5. Mycelial phase - delicate branching hyphae with ovoid conidia clustered at tip in "rosette" head or along side like a "sleeve"

Chromoblastomycosis

1. *Phialophora* and *Cladosporium* - foot or leg
2. Scaly, wart-like lesions
3. Brown pigmented hyphae
4. Characteristics
    a. *Cladosporium* (*C. carionii)* - conidia in branched chains
    b. *Phialophora* (*P. verrucosa*) - conidia produced in a flask-like conidiophore or phialide

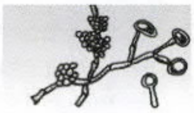
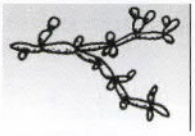
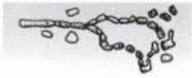
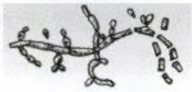
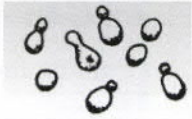
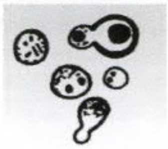
c. Acrotheca (*Rhinocladiella-like*) (*Fonsecaea pedrosoi*) - conidia formed along side of irregular, club shaped conidiophores; this genus exhibits all three types of sporulation

TEST Alert!
I.D. Dimorphic Fungi from Graphic Images & Culture Descriptions

Mycetoma

1. Found in the tropics
2. Foot trauma; draining sinuses
3. Purplish discoloration and tumor-like deformities that drain pus with granules
4. Actinomycotic - *Nocardia, Actinomadura*
5. Fungal
   a. *Pseudallescheria boydii* (perfect stage)
   b. *Scedosporium apiospermum* (*imperfect stage*)
6. White cottony mycelium; turns brown with age; oval conidia borne on conidiophores ("*lollipop*")
7. May also be found in eye, sinuses, brain abscess
8. Opportunist in compromised patients

YEASTS AND OTHER OPPORTUNISTS

Organism	Characteristics	Notes	Illustrations
Candida albicans	Numerous Blastoconidia Along Pseudohyphae; Germ Tubes Formed; Terminal Chlamydoconidia	Germ Tube Test - Pos in 2 Hrs. at 37C in Rabbit or Human Plasma; Urease Neg; May be Isolated in Blood of Immunosuppressed	
Candida tropicalis	No Chlamydoconidia Sparser Blastoconidia	Germ Tube Test - Neg (But, Forms Structure Between Tube & Spore)	
Geotrichum	Arthroconidia	"Hockey Stick" Bud on One Corner of Arthroconidia	
Trichosporon	Arthroconidia and Blastoconidia	Budding from Both Corners of Arthroconidia Urease Pos	
Candida (torulopsis) *glabrata*	No Pseudohyphae	Assimilates Only Glucose & Trehalose	
Cryptococcus neoformans	No Pseudohyphae; Encapsulated; India Ink Pos	Urease Pos; Brown Colonies on Birdseed Agar; May be Isolated in Blood of Immunosuppressed	

Microscopic Observations

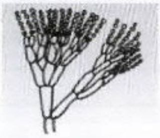
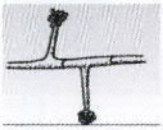
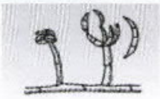
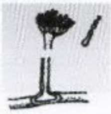
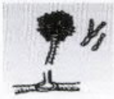
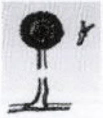
Description	Possible Organism
Small Extracellular Yeast	*Candida sp.* *Sporothrix schenckii*
Small Intracellular Yeast	*Histoplasma capsulatum*
Yeast with Capsule	*Cryptococcus neoformans*
Yeast with Pseudohyphae	*Candida sp.*
Large Yeast with Broad-Based Buds	*Blastomyces dermatitidis*
Large Yeast with Multiple Buds	*Paracoccidioides brasiliensis*
Endospherules and Endospores	*Coccidioides immitis*

TEST Alert!

I.D. Yeast from Graphic Images

Yeasts Found in Blood Cultures

Opportunists

Organism	Characteristics	Illustrations/Notes
Penicillium	Green or Blue-Green Colonies; Branching or "Penicillus" Head; Sterigmata Blunt	
Acremonium	Delicate Hyphae; Elliptical Conidia with Appearance of Brain Surface	
Fusarium	Colonies Lavender to Purple; "Banana" - Shaped Macroconidia	
Aspergillus	Conidiophore Ends in Swelling ("Vesicle")Which Carries Sterigmata and Chains of Conidia	Respiratory Infections "Farmer's Lung"
fumigatus	Green Conidia	
flavus	Yellow Conidia	
niger	Black Conidia	

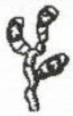
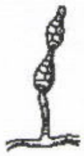
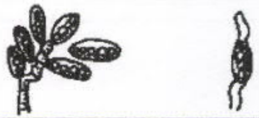
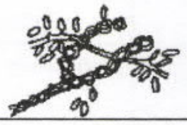
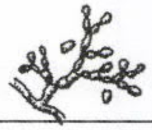
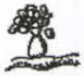
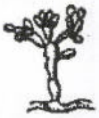
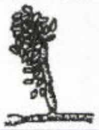
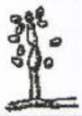
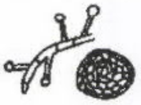
TEST Alert!

I.D. Most Common Opportunists from Graphic Images

TEST Alert!

Differentiate Penicillium and Aspergillus from Graphic Images

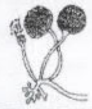
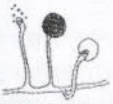
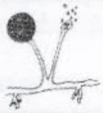
Dematiaceous Molds

Organism	Characteristics	Notes	Illustrations
Curvularia	Macroconidia - 4-6 Cells; Center Cell Larger; Gives a Curved Appearance	Saprobe	
Alternaria	Macroconidia Have Transverse and Longitudinal Septa in Chains	Saprobe	
Bipolaris	Similar to Drechslera; Slightly Protruding *("Squared-Off" Hilum)*	Germ Tube Forms along Conidial Axis	
Aureobasidium	Produces White Yeast-Like Colonies and Black Yeast-Like Colonies	Large, Segmented Hyphae; Appear as Oreo® Cookies	
Cladosporium	Branching Conidiophores with Chains of "Tree-Like" Conidia	Cladosporium-Type Sporulation; Shield Cells; Scars Where Conidia Attach	
Phialophora verrucosa	Flask-Shaped Phialides With a Distinct Collarette at the Apex; Conidia in Clusters at Apex	Phialophora-type Sporulation; Looks Like "Vase of Flowers"	
Fonsecaea pedrosoi	Short Chains of Conidia from Sides of Conidiophores *(Initial Appearance - "Rabbit Ears")*	Acrotheca or Rhinocladiella-Type Sporulation; May See Cladosporium or Phialophora-Type Sporulation	
Exophiala jeanselmei	Initially Form Black Yeast Cells; Eventually Form Hyphae	Forms Conidiophores Which Terminate in Tapered Annelids; Does NOT Grow at 40C	
Wangiella dermatitidis	Initially Form Black Yeast Cells; Eventually Form Hyphae	Opposite of Above	
Pseudallescheria boydii (Perfect or Sexual Form)	"Lollipop" Forms; Cleistothecia *(Only in Sexual Form)*	*Scedosporium apiospermum (Asexual or Imperfect Form)*; Can be Confused with *Blastomyces (Mycelial Phase)*	

TEST Alert!

I.D. Black Mold from Graphic Image or Description

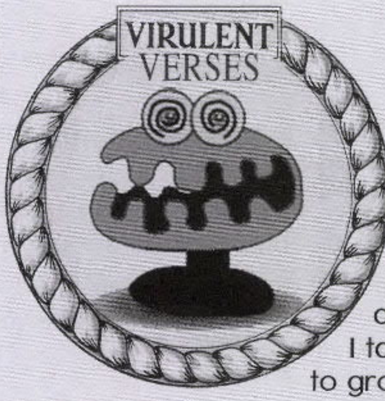
Phycomycetes

Organism	Characteristics	Illustrations
Rhizopus	Nodal Rhizoids; Sporangiophore Ends in Swelling (*"columella"*)	
Mucor	No Rhizoids	
Absidia	Internodal Rhizoids	

REMEMBER!

Only the Curious
Will Learn . . .

— Eugene S. Wilson



Candida albicans

Candida albicans
is one bad yeast
BAP or SAB will
grow the beast.

In the mouth or
down below,
I take every chance
to grow.

I live in your gut or gums
And look like *staph* when I'm young.

With pseudohyphae, out I stretch
to spread the pain and the itch.

Look for chlamydoconidium
And find germ tube production.

MYCOLOGY SAMPLE QUESTIONS

1. A fungal specimen isolated from the bone marrow of a patient with a pulmonary infection showed tuberculate macroconidia at 30C and yeastlike cells at 36C. Identify the most likely dimorphic fungi with these characteristics.
   - A. *Aspergillus fumigatus*
   - B. *Blastomyces dermatitidis*
   - C. *Histoplasma capsulatum*
   - D. *Trichophyton mentagrophytes*

2. A yeast-like organism was isolated from a sputum specimen. On cornmeal agar, this yeast produced mycelia with thick-walled terminal chlamydoconidia. This organism is most likely
   - A. *Blastomyces dermatitidis*
   - B. *Candida albicans*
   - C. *Candida tropicalis*
   - D. *Geotrichum candidum*

3. Thick-walled yeast cells bearing single buds attached by a broad base are observed in an aspirated clinical specimen. The organism is most likely
   - A. *Blastomyces dermatitidis*
   - B. *Candida albicans*
   - C. *Cryptococcus neoformans*
   - D. *Geotrichum candidum*

4. Crust from a cauliflower-like lesion on the hand microscopically exhibited brown spherical bodies. After 3 weeks incubation at room temperature, a black mold grew on Sabou-raud's agar. Microscopic examination revealed cladosporium, phialophora and acrotheca sporulation. The most probable identification is
   - A. *Cladosporium carrionii*
   - B. *Fonsecaea pedrosoi*
   - C. *Phialophora verrucosa*
   - D. *Pseudallescheria boydii*

5. In order to prove a yeast is dimorphic, which of the following tests is performed?
   - A. Carbohydrate assimilation
   - B. Growth on cornmeal agar
   - C. Incubate yeast subculture at 37C
   - D. Urease

ANSWERS AND RATIONALE

1. C

Option A causes "Farmer's Lung" but is NOT a dimorphic fungus and is usually identified from a sputum sample (*branching hyphal filaments in a characteristic Y shape*). Option B is a dimorphic fungus but, as a mold, it bears spherical conidia from the sides of the hyphae frequently appearing in "lollipop" forms. It is usually identified from a sputum sample or may spread via thebloodstream and infect the skin (*identified from a skin scraping*). Option D is a dermatophyte and NOT a dimorphic fungus and is identified from skin, hair or nail scrapings.

2. B

Option A is a dimorphic fungus and as a yeast produces broad-based buds with a double contoured wall. Option C does not produce chlamydoconidia. Option D produces characteristic rectangular arthroconidia.

3. A

Option B produces small oval yeasts which may be single cells or often appear with buds, hyphae or pseudohyphae (*elongated yeast cells that remain attached to each other*). Option C is a yeast which forms a large capsule (*seen on India ink preps*) and may be isolated from spinal fluid. Option D produces a "hockey stick" bud on rectangular arthroconidia.

4. B

F. pedrosoi is the only mold which produces all three types of sporulation.

5. C

Dimorphic fungi demonstrate yeast forms at 36C and mycelial (*mold*) forms at 28-30C. Option A identifies yeast isolates. Option B (*with Tween 80)* allows for enhanced formation of hyphae, blastospores and chlamydospores. Option D is positive with many yeasts (*C. albicans is NEGATIVE for urease while C. neoformans is positive*).

PARASITOLOGY

by Michele Zitzmann and Mary R. Hebert

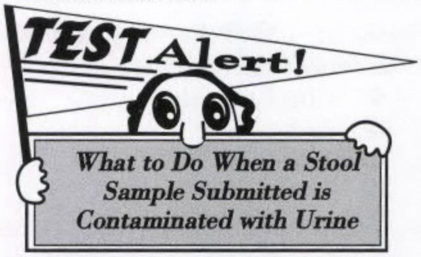
Specimen Collection and Handling

GENERAL

1. Recommended to examine 3 specimens within 10 day span; every other day if possible
2. Examine liquid specimens within 30 minutes of passage or place in preservative
3. Examine soft specimens within 1 hour of passage or place in preservative
4. Examine formed stools within 24 hours; place an aliquot in preservative and refrigerate the remainder

TYPES OF SPECIMENS

1. Feces - 95% of parasite specimens
    a. Collect in clean, dry container with a secure lid
    b. Do not accept specimens contaminated with urine (*may destroy motile organisms*), water (*may contain free living organisms*) or oil or barium enemas (*intestinal protozoa may be undetectable 5-10 days after barium is given)*
    c. Antibiotics (*such as tetracycline*) modify intestinal flora and may prevent parasite recovery for 2 weeks after drug cessation
2. Sputum - early morning specimen is best (*most concentrated*)
3. Urine - early morning or 1st void
4. Genitalia - saline wet swabs
5. Tissue and skin - sterile container
6. Blood
    a. Fresh blood from fingerstick (*preferred over EDTA tube of blood)*
    b. Prepare thick smear for concentration and thin smear for identification


TEST Alert!
What to Do When a Stool Sample Submitted is Contaminated with Urine

Specimen Collection and Handling for Ova, Cyst and Parasite Examination

Ova, Cyst & Parasite Examination

Macroscopic →

Consistency: Liquid- trophozoites
Soft- trophozoites & cysts
Formed- protozoan cysts (*helminth eggs may be found in any type of stool specimen*)

Presence of: Mucus, blood or macroscopic parasites

Color: Black- blood / iron
Light tan/ white- barium or absence of bile

Microscopic →

May reveal wide variety of findings (parasites, RBCs, WBCs such as eosinophils, Charcot-Leyden crystals, fungi such as *Candida sp.*)

Permanent Smears →

Trichrome Stain- Easy to perform; most commonly used
Wrights's stain and Giemsa stain- used for blood smears
Acid Fast Stain- used to diagnose Cryptosporidium, Isopora, Cyclospora
Modified Trichrome Stain- used to diagnose microsporidiosis
Iron Hematoxylin Stain-can enhance detection of microfiliarial sheath

Wet Mounts →

Saline (0.85% NaCl)- detects motility; must use low light
Iodine- Kills trophozoites; enhances nuclear material
Not performed on preserved specimens

TYPES OF PRESERVATION

1. Refrigeration - good for eggs, larvae and amoebic cysts; **Do not refrigerate if you suspect amoebic trophozoites**

2. 10% formalin - good for eggs, larvae and amoebic cysts

3. MIF (*Merthiolate-Iodine-Formalin*) concentration procedure as well as preservative; good for eggs larvae and amoebic cysts

4. PVA (*Polyvinyl Alcohol)* best for amoebic trophozoites; can prepare permanent stain slides from specimens preserved this way

5. SAF (*Sodium Acetate-Acetic Acid-Formalin)* good for amoebic trophozoites; environmentally safer than PVA

6. Schaudinn's Fluid-used for fresh stool samples;good for trophs and cysts

CONCENTRATION TECHNIQUES: (USED TO DETECT SMALL NUMBERS OF PARASITES)

1. Formalin-Ethyl Acetate Sedimentation:
   a. Forms four layers:
      - ❖ *Ethyl acetate*
      - ❖ *Debris/ fat*
      - ❖ *Formalin*
      - ❖ *Sediment (parasites);*

   b. Ethyl acetate removes fats & oils Formalin preserves organisms
   c. Advantages: Can stay in formalin stage indefinitely; easy to perform; detects all parasites
   d. Disadvantages: more debris

2. Zinc Sulfate Flotation Technique
   a. Specific gravity of zinc sulfate is greater than ova, cysts and larvae, so they float on top of the zinc sulfate solution
   b. Specific gravity of zinc sulfate solution should be 1.18
   c. Advantages - not flammable; produces a cleaner preparation
   d. Disadvantages - large eggs *(schistosomes*) and operculated eggs (*D. latum)* are often missed

MODES OF TRANSMISSION

1. Ingestion - eggs, cysts or larvae; examples:
   a. *Ascaris*
   b. *Paragonimus*
   c. *Trichinella*
   d. *Giardia*

2. Penetration - larvae penetrates directly through the skin; examples:
   a. *Strongyloides*
   b. Hookworm

3. Vector - vectors inject parasites into blood/tissue; examples:
   a. Mosquito -*Plasmodium, Brugia, Wuchereria*
   b. Tse Tse fly -*Trypanosoma*
   c. Tick- *Babesia*

4. Sexually
   a. *Trichomonas*

5. Blood Transfusion
   a. *Trypanosoma*
   b. *Plasmodium*
   c. *Babesia*

Helminths

TERMS

1. Intermediate host - host which contains the larval form of the parasite

2. Definitive host - host which contains the adult sexual form of the parasite

3. Hermaphroditic - contain both sexes in one helminth; cestodes and trematodes (*except Schistosomes)*

4. Gravid proglottid - segments filled with eggs

Intestinal Nematodes (Roundworms)

GENERAL CHARACTERISTICS

1. Males smaller than females and have a curved tail

2. Unsegmented

3. Length varies from a few millimeters to meters in length

4. Complete digestive tract (*mouth to anus*)

5. Worldwide distribution

Ascaris lumbricoides (Large Intestinal Roundworm)

1. Largest nematode

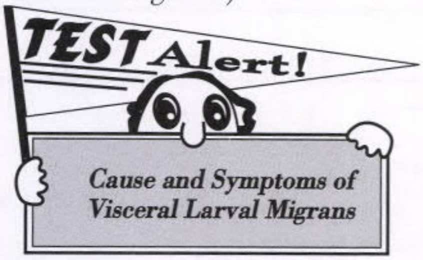
2. Second most common nematode infection in U.S.; 5-9 year old group most prevalent in U.S.; infective eggs ingested

3. Clinical disease
   a. "worm ball" - blockage in intestines
   b. "ascaris pneumonitis" - due to larvae migration in lungs

4. Diagnosis
   a. Demonstrate adult worms or characteristic egg in feces *(round egg containing albuminous coating)*; "decorticated" if egg loses coating
   b. Larvae in sputum or gastric washings

5. If no male present in small intestine, female will lay unfertilized eggs

6. *Toxocara canis/cati*- dog and cat ascarid; migration through tissues resulting in eosinophilia (*Visceral Larval Migrans*)



Cause and Symptoms of Visceral Larval Migrans

Enterobius vermicularis (Pinworm)

1. Most common helminth parasite of humans; frequently in children; infective eggs ingested

2. Symptoms include pruritis *(intense perianal itching)*

3. Diagnosis
   a. Scotch tape (*cellulose tape*) preparation - eggs and larvae stick to tape; since migration of female occurs at night the prep is performed after patient has been sleeping or early in the morning
   b. Adults may become "stuck" to the outside of the stool as it passes the perianal folds where the female migrates to lay eggs

Autoinfection Associated with Pinworms

4. Eggs become embryonated within hours resulting in a high rate of autoinfection; treat entire family of an infected individual

Trichuris trichiura (Whipworm)

1. Clinical disease — prolapsed rectum may occur in heavy infections; infective eggs ingested

2. Diagnosis - demonstrate characteristic egg in feces *(football shaped with mucoid polar plugs)*

Strongyloides stercoralis (Threadworm)

1. Smallest nematode

2. Rhabditiform larva (*noninfective*)
   a. Short buccal cavity (*approximately 1/3-1/2 width of body)*
   b. Prominant primordial genitalia

3. Filariform larva (*infective*)-penetrates skin
   a. Short buccal cavity
   b. Notch at end of tail

4. Eggs hatch in mucosa of intestine; rarely seen in feces

5. Has both a free living cycle and a parasitic cycle

6. Autoinfection - some of the rhabditiform larvae develop into filariform larvae in the bowel and reinfect the host

7. Clinical disease
   a. 3 stages based on life cycle
      - ❖ *Cutaneous - initial skin penetration*
      - ❖ *Pulmonary - larval migration through lungs*
      - ❖ *Intestinal - symptoms depend on worm load; immunocompromised patients may exhibit leukocytosis and eosinophilia*
   b. Hyperinfection syndrome- may lead to death from tissue damage
      - ❖ *Occurs in the immunocompromised (AIDS, drugs)*
      - ❖ *Can be transferred through organ transplantation*

8. Diagnosis - demonstrate rhabditiform larvae and/or filariform larvae in feces

Infective Stage of Strongyloides

Treatment in AIDS Must be Prolonged & May be Unsuccessful

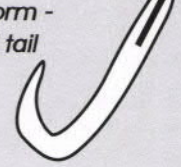
Necator americanus (New World Hookworm) and Ancylostoma duodenale (Old World Hookworm)

1. Adults
   a. May live 2-14 years
   b. Rarely seen in stools since firmly attached to mucosa
2. Rhabditiform larva
   a. Long buccal cavity (*approximately as long as width of body)*
3. Filariform larva - (infective stage, penetrates skin)
   a. Long buccal cavity
   b. Pointed tail
4. Clinical disease
   a. Pneumonitis
   b. Allergic reactions - "ground itch"
   c. Anemia - each adult worm consumes 0.2 ml of blood/day
5. Diagnosis
   a. Demonstrate characteristic egg in feces
   b. Do not see larvae in feces (*unless specimen left at room temperature & eggs hatch*)
6. *Ancylostoma braziliensis/caninum*- dog and cat hookworm; migration through tissues causing linear tracts; (*Cutaneous Larval Migrans or "creeping eruption"*)

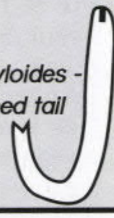
REMEMBER!
Hookworm vs.
Strongyloides: Larvae

RHABDITIFORM:
STRONGYLOIDES- short and sexy (*short buccal cavity and presence of genitalia*)

FILARIFORM:
- *Hookworm - pointed tail*

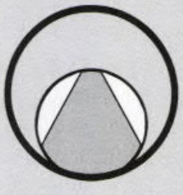


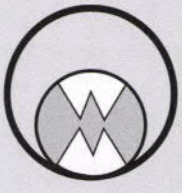
- *Strongyloides - notched tail*



REMEMBER!
Differentiating Hookworm Adults

• ***NECATOR** - **Cutting Plates***



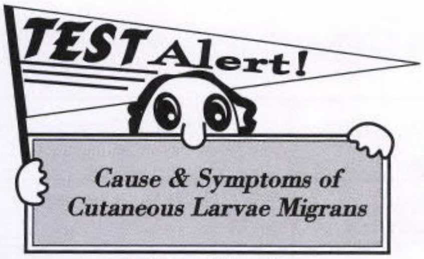


•***ANCYLOSTOMA** - **Teeth or DUODENALE - 2 pair***

BLOOD AND TISSUE NEMATODES

Trichinella spiralis

1. Adults
   a. Females bear larvae **NOT** eggs
2. Infective stage - ingestion of encysted larvae in undercooked pork
3. Clinical disease
   a. Symptoms initially resemble food poisioning
   b. Destruction of muscle cell (*especially active muscles)*
   c. High eosinophilia (*may reach 90%)*
   d. Myocardial involvement possible
4. Diagnosis - muscle biopsy showing encysted larvae in striated muscle; serological testing



Cause & Symptoms of Cutaneous Larvae Migrans

Microfilariae

GENERAL CHARACTERISTICS

1. Require an arthropod as an intermediate host; when infected arthropod takes a blood meal, the microfilariae are released into human host
2. Diagnosis made by examining Giemsa stained thick and thin smears (*except Onchocerca volvulus - skin scraping from nodules)*

Wuchereria bancrofti

1. Microfilaria (*larval stage*)
   a. Sheathed but NO nuclei in tip of tail
   b. Nocturnal periodicity — 9 pm-2 am greatest concentration in blood
2. Elephantiasis - permanent blockage of lymphatic system; usually occurs in the lower extremities and genitalia
3. Diagnosis - demonstrate larvae in blood smear

Brugia malayi

1. Microfilaria
   a. Sheathed
   b. 2 terminal nuclei at tip of tail; separate from rest of body nuclei
   c. Nocturnal periodicity
2. Elephantiasis restricted to the lower extremities
3. Diagnosis - demonstrate larvae in blood smear

Loa Loa (Eye Worm)

1. Microfilaria
   a. Sheathed with nuclei to the tip of tail
   b. Diurnal periodicity — 10 am-3 pm greatest concentration in blood
2. Causes calabar swellings (*allergic reaction to worm migration in tissue)*
3. Diagnosis
   a. Demonstrate larvae in blood smear
   b. Worm may migrate across conjunctiva

Onchocerca volvulus

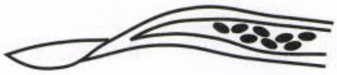
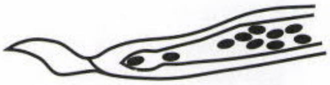
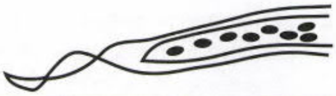
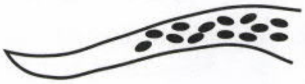
1. Microfilaria
   a. Only pathogenic microfilaria which is NOT sheathed
   b. NO nuclei in tip of tail
   c. Found in nodules under skin, NOT in peripheral blood
2. Clinical disease
   a. Severe dermatitis (*50-70% eosinophilia)*
   b. Onchocercoma-nodule containing encapsulated adult
   c. Microfilariae in ocular structures may result in blindness; leading cause of blindness in Africa (*onchocerciasis; river blindness*)
3. Diagnosis - demonstrate larvae in skin "snips"/tissue scrapings

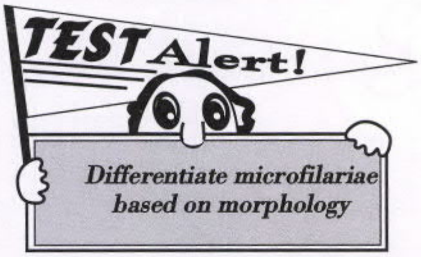
REMEMBER!

Capt. Mic Ro Filariae

- ***ONCHOCERCUS VOLVULUS*** **-**
  Only pathogenic microfilariae with no sheath, no nuclei in tip of tail (*"on" is "no" backwards*)
- ***WUCHERERIA BANCROFTI*** **-**
  No nuclei in tip of tail
- ***BRUGIA MALAYI*** **-**
  B is second letter of alphabet (*2 nuclei in tip of tail*)
- ***LOA LOA*** **-**
  Name repeats and so do nuclei, continuously to tip of tail

Characteristics of Microfilariae

Microfilaria	Disease	Arthropod Vector	Diagnostic Stage Found In:	Diagnostic Characteristics
Wuchereria bancrofti	Elephantiasis	Mosquito (Culex & Anopheles)	Blood	Sheath, no nuclei in tail 
Brugia malayi	Elephantiasis	Mosquito (Mansonia)	Blood	Sheath, 2 nuclei in tip of tail 
Loa loa	Calabar Swellings Blindness	Fly (Chrysops)	Blood	Sheath, continuous nuclei in tail 
Onchocerca volvulus	Blindness onchocercoma (nodule)	Fly (Simulian)	Tissue from Nodule	No sheath, no nuclei in tail 



Differentiate microfilariae based on morphology

Infective and Diagnostic Stages of Nematodes

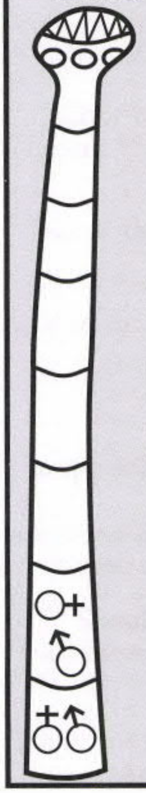
CESTODES (TAPEWORMS)

General

1. Flat, ribbon-like, segmented worms
2. Shape of proglottids, presence or absence of armed rostellum and size aid in identification of adults
3. Hermaphroditic - mature proglottids contain both male and female reproductive organs
4. 4 cup shaped suckers on scolex (*except Diphyllobothrium latum which has 2 suctorial grooves)*

REMEMBER!

Cecil / Cecily The Hermaphroditic Tapeworm



T ***taenia species (example)***
A ***adult - small intestine***
P ***proglottids contain male & female parts***
E ***eggs - 6 hooked embryos (hexacanth)***
W ***worldwide***
nO ***respiratory, vascular or digestive systems***
R ***ribbon like body, flat***
M ***middle (intermediate) host required***

Taenia saginata (Beef Tapeworm)

1. Adults
   a. Scolex has an unarmed rostellum
   b. Consists of as many as 2000 proglottids (*10-15 ft long*)
   c. May live 25 years
2. Infective stage - ingestion of undercooked beef containing larval stage
3. Diagnostic stage
   a. Find characteristic egg in feces; resembles kiwi slice
   b. Proglottids can be stained; note number of major uterine branches *(15-30 in T. saginata)*

Taenia solium (Pork Tapeworm)

1. Adults
   a. Scolex has armed rostellum
   b. Consists of as many as 1000 proglottid (*6-10 ft long*)
2. Infective stage - ingestion of undercooked pork containing larval stage
3. Cysticercosis - human is intermediate host
   a. Man ingests the egg of *T. solium*
   b. Egg passes through the stomach and hatches in the intestine
   c. The embryo penetrates the mucosa and becomes a cysticercus; most commonly found in the subcutaneous connective tissue, eye, brain, muscles, heart and lungs
4. Diagnostic stage
   a. Find characteristic egg in feces (*identical to T. saginata)*
   b. Proglottids can be stained; note number of major uterine branches *(7-13 in T. solium)*

Differentiating Taenia Species

T. solium	*T. saginata*
Pork	Beef
7-13 Uterine Branches	15-30 Uterine Branchesl
Armed	Unarmed
Cysticercosis	Rare Cysticersosis

REMEMBER! Taenia Species

T. solium

- "soul" food = pork
- "soul" sister = cysti(*sister*)cercosis
- parasite causing this serious condition must be armed

T. saginata

- has more letters in its name and more uterine branches

TEST Alert!

Differentiate Proglottids of T. saginata and T. solium

Intermediate Host (man) & Infective Stage (T. solium egg) in Cysticercosis

Hymenolepis nana (Dwarf Tapeworm)

1. Most common human tapeworm in the U.S.
2. Adults
   a. Small tapeworm; 40 mm long
   b. Contains approximately 200 segments
   c. Armed rostellum *(short with hooks)*
3. Does not require an intermediate host
4. Infective stage - ingestion of eggs
5. Diagnostic stage - demonstrate characteristic egg in feces; eggs contain polar filaments
6. Heavy infections can occur through autoinfection

Hymenolepis diminuta (Rat Tapeworm)

1. Adult - unarmed rostellum
2. Man is accidental host via ingestion of infected intermediate arthropod host *(Ex. grain beetles in pre-cooked cereals)*
3. Rat is definitive host
4. Infective stage - ingestion of beetle/ flea
5. Diagnostic stage - demonstrate characteristic egg in feces; eggs look similar to *H. nana*, except lack polar filaments

Diphyllobothrium latum (Broad Fish Tapeworm)

1. Adults
   a. Scolex consists of two longitudinal suctorial grooves known as bothria, giving it a spoon shape
   b. Uterus in gravid proglottid appears as a rosette
   c. May have as many as 3000 proglottids
2. Infective stage - ingestion of larvae in infected undercooked freshwater fish
3. Diagnostic stage - demonstrate characteristic egg or proglottids (*often in chains of a few inches to several feet)* in feces *(may NOT be seen in flotation techniques; operculated ova may sink)*
4. Only cestode to produce operculated eggs
5. Egg possesses a small abopercular knob

6 Clinical disease
   a. Can cause megaloblastic anemia, since vitamin B_{12} is absorbed by worm
   b. Sparganosis - disease caused by drinking water containing infected copepod; larva develops in human who is the intermediate host instead of the fish
   c. High incidence in Finland, Alaska, and Canada

Echinococcus granulosus - (Hydatid Tapeworm)

1. Adult
   a. Very small; 3-6 cm long
   b. Consists of only three proglottids - immature, mature and gravid
   c. Scolex has an armed rostellum
2. Normal life cycle
   a. Sheep (*intermediate host*) ingest eggs
   b. Dog (*definitive host)* infected from eating infected viscera of butchered animals
3. Infective stage - man (*intermediate host)* ingests egg (*close contact with dog*)

Hydatid Disease - Man Ingests Egg & Becomes Intermediate Host

4. Diagnostic stage
   a. Hydatid cysts seen in routine x-rays or exploratory surgery *(form in various parts of the body - most commonly the liver, lungs, brain, heart)*
   b. Serological tests (*ELISA, IHA)*

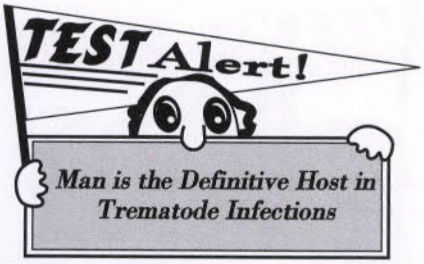
Dipylidium caninum - (Dog Tapeworm)

1. Adults
   a. Scolex has an armed rostellum
   b. Proglottids resemble pumpkin/ cucumber seeds when moist and rice grains when dry
2. Normal life cycle - dog and cat ingest infected fleas containing larvae
3. Infective stage - human (*accidental intermediate host)* ingests infected flea
4. Diagnostic stage - demonstrate characteristic egg packet (*5-30 eggs are in a hyaline non- cellular egg sac)* in feces
5. Found predominantly in children

Trematodes (Flukes)

GENERAL

1. Flat leaf-shaped organisms
2. Hermaphroditic - contain both male and female reproductive parts (*except Schistosomes)*
3. Require an intermediate host
4. Snail is always 1st intermediate host
5. Eggs are operculated (*except Schistosomes*)



Man is the Definitive Host in Trematode Infections

Fasciolopsis buski - (Giant Intestinal Fluke)

1. Infective stage - ingestion of raw aquatic vegetation (*Ex. water chestnuts*) with encysted metacercaria
2. Diagnostic stage - detect characteristic eggs in feces *(eggs resemble F. hepatica); very large egg with small operculum*
3. Clinical Disease
   a. Diarrhea, epigastric pain
   b. Symptoms relate to number of worms present
4. Found in Asia

Fasciola hepatica - (Sheep Liver Fluke)

1. Infective stage - ingestion of raw aquatic vegetation (*Ex. water chestnuts*) with encysted metacercaria
2. Diagnostic stage - detect characteristic eggs in feces *(eggs resemble F. buski)*
3. Clinical disease
   a. Larvae elicit inflammatory response in liver during migration
   b. Obstructive jaundice, fever, vomiting, diarrhea, eosinophilia
4. Sheep and cattle are reservior hosts; therefore, high incidence in sheep raising countries

Clonorchis sinensis - (Chinese Liver Fluke)

1. Infective stage - ingestion of raw fish infected with metacercaria
2. Diagnostic stage - demonstration of characteristic eggs in feces (*operculated with shoulders and small comma-shaped appendage at abopercular end*)
3. Found in Japan, Korea, China, Taiwan and Vietnam

Paragonimus westermani (Oriental Lung Fluke)

1. Infective stage - ingestion of raw crustacea (*crabs, crawfish, etc.)* infected with metacercaria
2. Diagnostic stage - demonstrate characteristic egg in feces or sputum (*may appear macroscopically in sputum as reddish-brown flecks resembling iron filings); (operculated with shoulders and thick abopercular shell)*
3. Clinical disease
   a. Light infections asymptomatic
   b. High eosinophilia
   c. Chronic cough and abundant mucus in heavy infections
4. Found in Japan, Korea, China, Philippines, and Southeast Asia

Blood Flukes (Schistosomes)

1. Most important trematode in man because of severity of infection
2. Separate male and female adult worms
3. Adult flukes live in venules *(S. japonicum and S. mansoni in mesenteric venules and S. haematobium in bladder venules)* and may live 4-35 yrs
4. Snail is intermediate host
5. Infective stage - cercariae in water directly penetrate skin of man *(definitive host)*
6. Diagnostic stage - demonstration of characteristic egg in feces or urine (*S. haematobium)*; may NOT be seen in flotation techniques (*large eggs may sink)*
7. Eggs possess a characteristic spine (*used to speciate)*
   a. *S. mansoni* = conspicuous lateral spine
   b. *S. japonicum* = inconspicuous lateral knob
   c. *S. haematobium* = terminal spine
8. Clinical disease
   a. Progressive chronic inflammatory disease involving liver, small intestine, large intestine and bladder

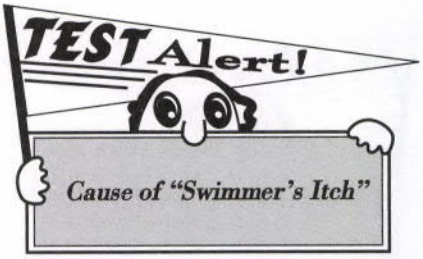
b. "Swimmer's Itch"- dermatitis due to skin penetration of cercariae from birds & other mammals; seen in the US
c. High eosinophilia
d. Hematuria (*S. haematobium*)
e. Bloody diarrhea (*S. mansoni* & *S. japonicum*)

9. Found in Western hemisphere (*transmission does not occur in U.S. because lack of appropriate snail)*

REMEMBER!

Helminth Names

Common name of worms give you clues to the distribution and/or location of adult worms. (*Ex. Clonorchis-Chinese Liver Fluke, adults found in the liver)*

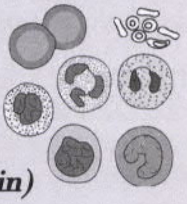


Cause of "Swimmer's Itch"

SCIENTIFIC NAME	COMMON NAME
Ancylostoma caninum	Dog hookworm
Ancylostoma duodenale	Old World hookworm
Ascaris lumbricoides	Large intestinal roundworm
Clonorchis sinensis	Oriental/Chinese liverfluke
Diphyllobothrium latum	Broad fish tapeworm
Dipylidium caninum	Dog tapeworm
Echinococcus granulosus	Hydatid tapeworm
Enterobius vermicularis	Pinworm
Fasciolopsis buski	Large intestinal fluke
Fasciola hepatica	Sheep liver fluke
Hymenolepis diminuta	Rat tapeworm
Hymenolepis nana	Dwarf tapeworm
Loa loa	Eyeworm
Necator americanus	New World hookworm
Onchocerca volvulus	Blinding worm
Paragonimus westermani	Oriental lung fluke
Schistosoma haematobium	Bladder fluke
Schistosoma japonicum	Oriental blood fluke
Strongyloides stercoralis	Threadworm
Taenia saginata	Beef tapeworm
Taenia solium	Pork tapeworm
Toxocara canis/cati	Dog/Cat Ascarid
Trichuris trichiura	Whipworm

REMEMBER!

Beyond Stools: Look for me in:



BLOOD

Microfilariae (larvae)
- except Onchocerca (skin)

URINE

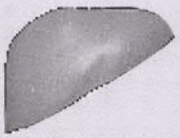
Enterobius vermicularis (eggs)
Schistosoma haematobium (eggs)

SPUTUM

Paragonimus westermani (eggs)
Ascaris lumbricoides (adults),
Strongyloides stercoralis (adults, larva, or eggs)

MUSCLES

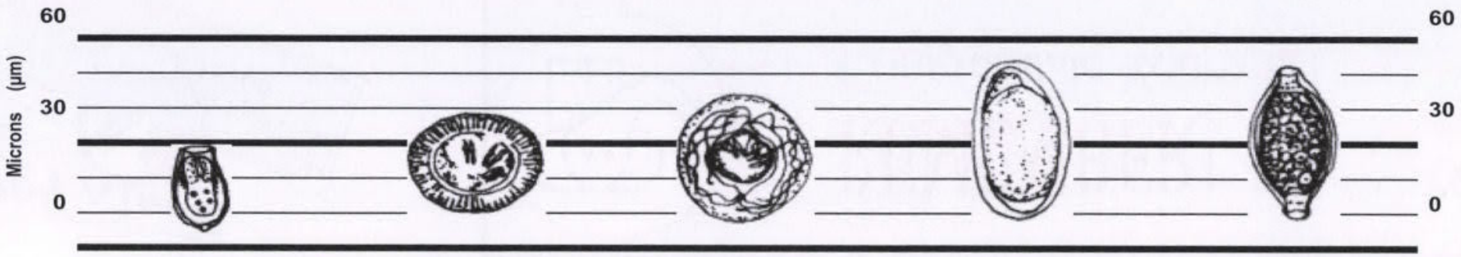
Trichinella spiralis (larvae)



LIVER / BILE FLUID

Echinococcus granulosus (larvae)
Clonorchis sinesis (adults)
Fasciola hepatica (adults)

Key Characteristics of Helminth Eggs



Clonorchis sinensis

- Lightbulb shape with small nob at abopercular end
- Operculated with shoulders
- Yellow-brown
- Contains miracidium

Taenia solium
Taenia saginata

- Round sunburst appearance due to outer striated layer (sliced kiwi)
- Oncosphere with six hooklets
- Cannot differentiate species

Hymenolepis nana

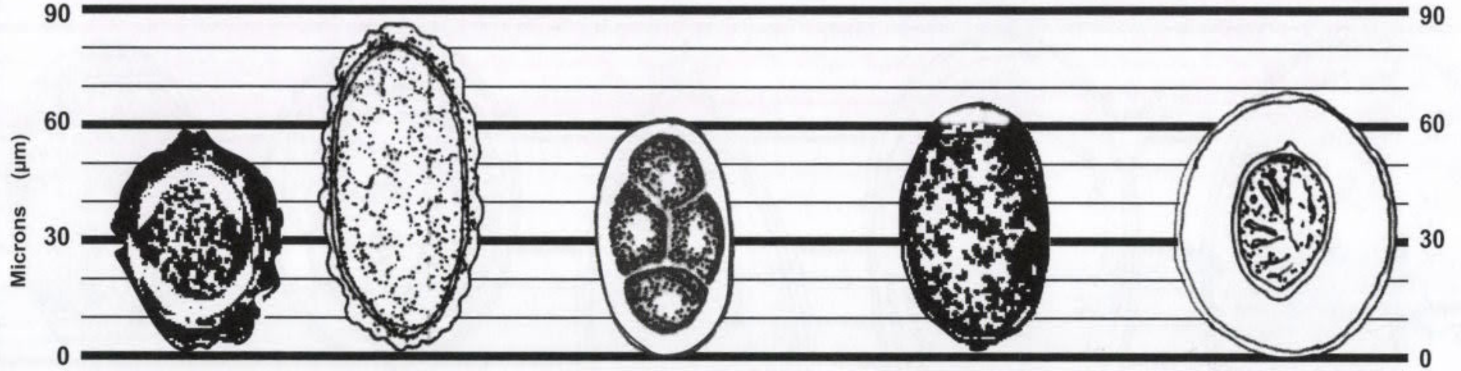
- Oval
- Oncosphere with six hooklets
- Bipolar
- Polar filaments
- Colorless

Enterobius vermicularis

- Elongated - oval (with one side flattened)
- Can become airborne and remain viable for years
- Embryonated within hours resulting in high rate of autoinfection

Trichuris trichiura

- Football / barrel shape with bipolar plugs
- Bile-stained (brown)



Fertile Infertile
Ascaris lumbricoides

- Oval to round in shape
- Outer layer is an albuminous coat (gives the egg a scalloped edge appearance)

Ancylostoma duodenale
Necator americanus
(Hookworms)

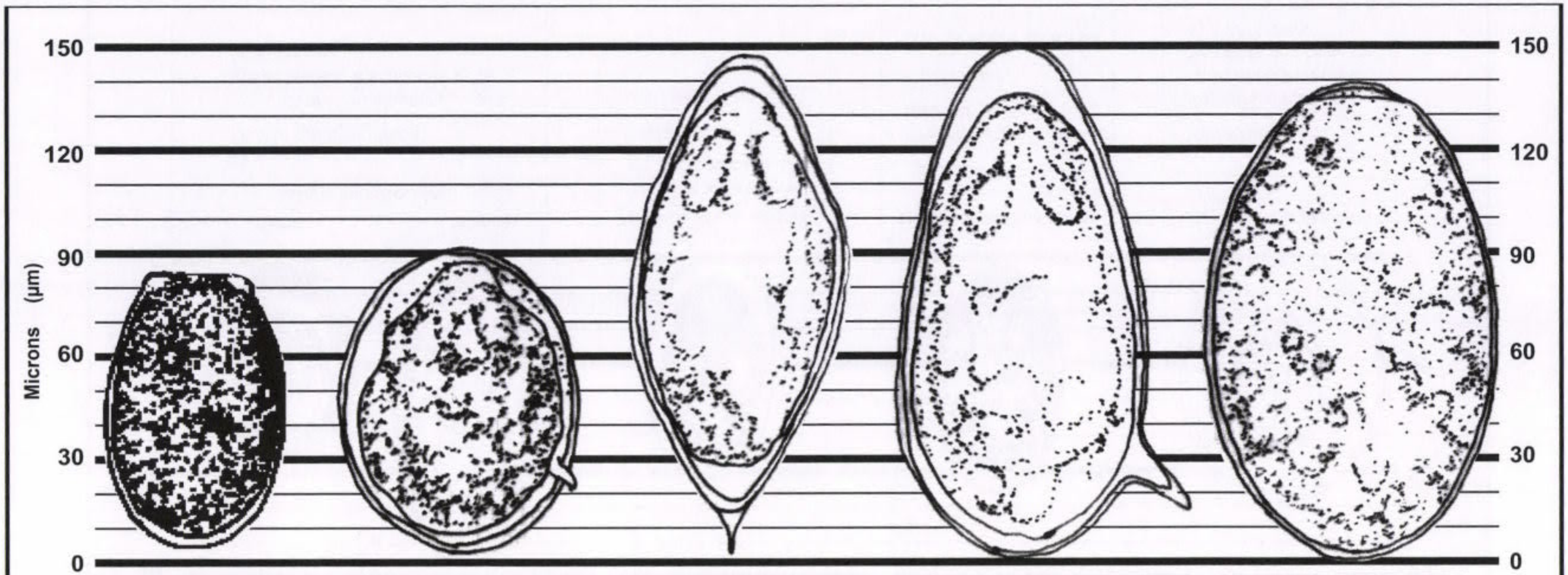
- Oval in shape
- Thin, smooth shell

Diphyllobothrium latum

- Broadly oval, very symmetrical
- Operculated
- Nob at abopercular end

Hymenolepis diminuta

- Round in shape
- Appears similar to H. nana except no polar filaments and twice the size of H. nana


Microns (µm)
150
120
90
60
30
0

Paragonimus westermani

- Large, broadly oval, non-symmetrical
- Operculated with shoulders; thickened end
- Found in feces or sputum

Schistosoma japonicum

- Round
- Small, rudimentary lateral spine / knob

Schistosoma haematobium

- Elongated
- Terminal spine
- Found in urine

Schistosoma mansoni

- Profile of a "man" !
- Elongated
- Large lateral spine

Fasciola hepatica
Fasciolopsis buski

- Difficult to differentiate the two
- Broadly elipsoidal
- Operculated

• Adapted with Permission from Morphologyof Diagnostic Stages of Intestinal Parasites of Humans; The Centers for Disease Control; Atlanta, GA 1989

TEST Alert!
Identify Helminth Eggs from Graphic Images

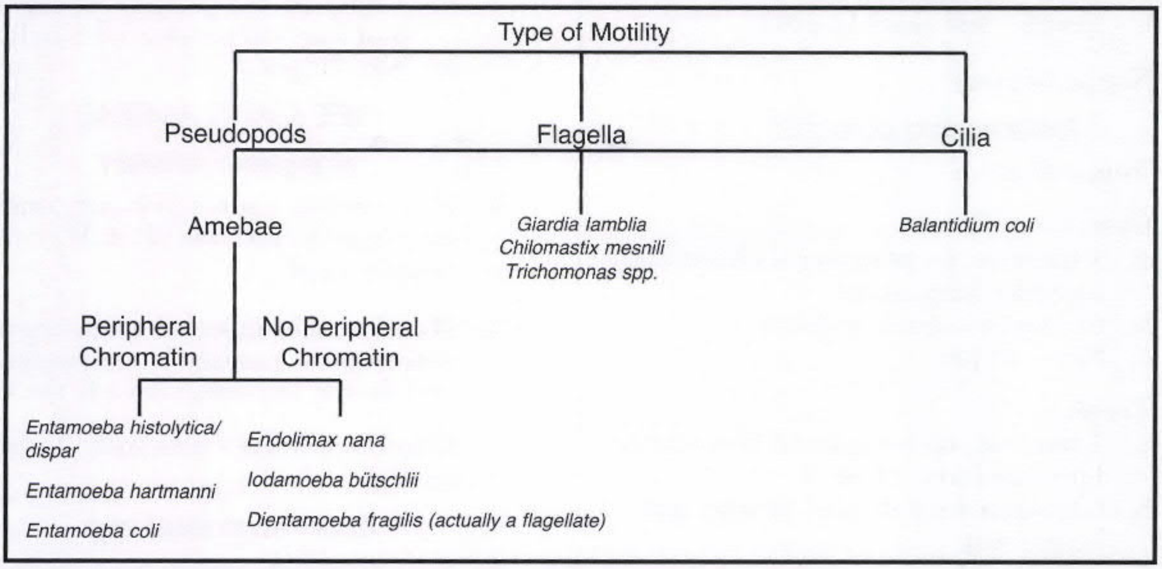
REMEMBER!

Congratulate Yourself !

You are almost through!!!

Protozoa

Classification of Intestinal Protozoa


Type of Motility
Pseudopods
Flagella
Cilia
Amebae
Giardia lamblia
Chilomastix mesnili
Trichomonas spp.
Balantidium coli
Peripheral Chromatin
No Peripheral Chromatin
Entamoeba histolytica/ dispar
Entamoeba hartmanni
Entamoeba coli
Endolimax nana
Iodamoeba bütschlii
Dientamoeba fragilis (actually a flagellate)

INTESTINAL AMEBAE

GENERAL CHARACTERISTICS

1. Motility by pseudopods
2. Infective stage - cyst (*usually ingested in contaminated food/water)*
3. Diagnostic stage is cyst or trophozoite (*troph*) in feces

Entamoeba histolytica

1. Pathogenic - causes ulcers of the intestinal tract; and liver or lung abscesses
2. Cyst
   - a. Up to 4 nuclei, uniform peripheral chromatin; central karyosome
   - b. May contain chromitoidal bars which are cigar-shaped
   - c. Size 10-20 µm
3. Troph
   - a. 1 nucleus, peripheral chromatin, central karyosome
   - b. May have ingested rbc's (*diagnostic*)
   - c. Size 12-60 µm
   - d. Progressive motility

Entamoeba dispar

1. Nonpathogenic
2. Morphologically identical to *E. histolytica*
3. Trophs cannot ingest red blood cells
4. Serological testing used to differentiate species

Entamoeba coli

1. Nonpathogenic
2. Confused with E. histolytica
3. Cyst
   - a. Up to 8 nuclei; irregular peripheral chromatin; eccentric karyosome
   - b. May contain chromatoidal bars which have splintered ends
   - c. Size 10-35 µm
4. Troph
   - a. 1 nucleus; peripheral chromatin; eccentric karyosome
   - b. Ingested bacteria, yeast
   - c. Sluggish motility
   - d. Size 15-50 µm

E. histolytica vs. E. coli

Central Karyosome Uniform Peripheral Chromatin		Eccentric Karyosome Irregular Peripheral Chromatin
Active, Progressive Motility May Have Ingested RBCs	**Troph**	Sluggish Motility May Have Ingested Bacteria
1,2, or 4 Nuclei Chromatoidal Bars *(*Cigar-Shaped*)*	**Cyst**	Up to 8 Nuclei Chromatoidal Bars (Pointed-Ends)

Entamoeba hartmanni

1. Identical appearance to E. histolytica except smaller
   a. Cyst: less than 10 µm
   b. Troph: less than 12 µm

2. Nonpathogenic

Iodamoeba butschlii

1. Nonpathogenic

2. Cyst
   a. 1 nucleus, no peripheral chromatin, blot-like karyosome
   b. Glycogen vacuole present
   c. Size - 12 µm

3. Troph
   a. 1 nucleus; no peripheral chromatin; blot-like karyosome
   b. Glycogen vacuole may or may not be present
   c. Size 15 µm

Endolimax nana

1. Nonpathogenic

2. Cyst
   a. 4 nuclei, no peripheral chromatin, blot-like karyosomes
   b. Clear halo around karyosomes
   c. Size 8 µm - smallest ameba

3. Troph
   a. 1 nucleus, no peripheral chromatin, blot-like karyosome with halo
   b. Size 10 µm

FREE LIVING AMEBAE

Naegleria fowleri

1. Pathogenic; causes primary amebic meningoencephalitis (*P.A.M.*) and is usually fatal

2. Man becomes infected when organism enters nasal passage while swimming and diving in ponds; invade the CNS

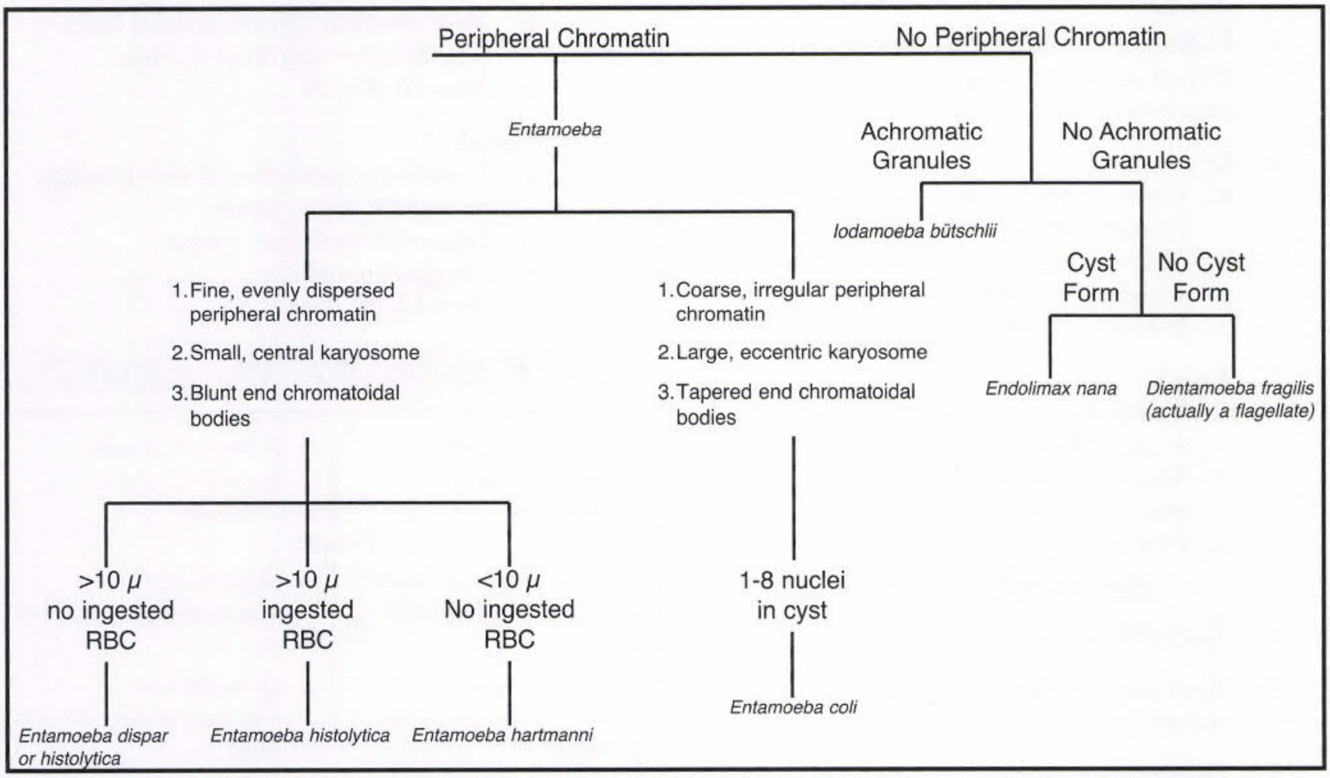
3. Diagnostic stage - demonstrate trophs in CSF

Acanthamoeba spp.

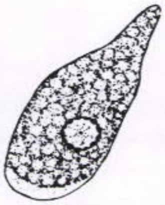
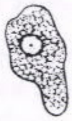
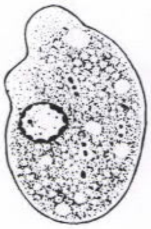
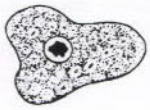
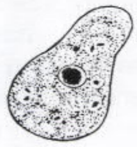
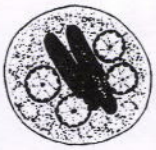
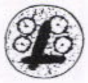
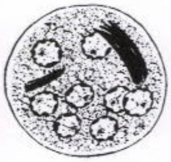
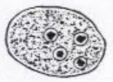
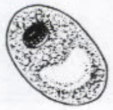
1. Usually affects immunocompromised patients

2. Causes keratitis and chronic form of meningoencephalitis; not usually associated with recreational water; usually trauma to the eye

Differentiating Amebae


Peripheral Chromatin
No Peripheral Chromatin
Entamoeba
Achromatic Granules
No Achromatic Granules
Iodamoeba bütschlii
Cyst Form
No Cyst Form
Endolimax nana
Dientamoeba fragilis (actually a flagellate)
1. Fine, evenly dispersed peripheral chromatin
2. Small, central karyosome
3. Blunt end chromatoidal bodies
1. Coarse, irregular peripheral chromatin
2. Large, eccentric karyosome
3. Tapered end chromatoidal bodies
>10 µ no ingested RBC
>10 µ ingested RBC
<10 µ No ingested RBC
1-8 nuclei in cyst
Entamoeba dispar or histolytica
Entamoeba histolytica
Entamoeba hartmanni
Entamoeba coli

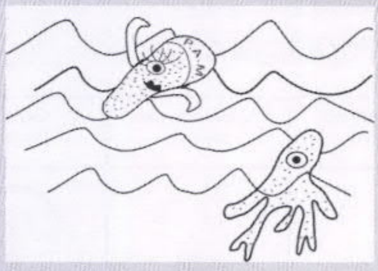
Amoeba Trophs and Cysts

	Entamoeba histolytica/dispar	Entamoeba hartmanni	Entamoeba coli	Endolimax nana	Iodamoeba bütschlii
Trophozoite					
Cyst					

Brooke MM, Melvin DM. *Morphology of Diagnostic Stages of Intestinal Parasites*, 2nd ed., CDC, 1984

REMEMBER!

Free Living Amebae



P.A.M.

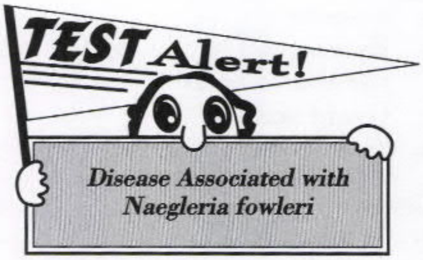
★ **to "foul" fresh water**

(Naegleria fowleri)

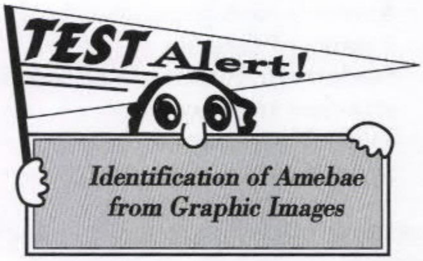
Kerry,

Acanthamoeba

causes Keratitis; associated with trauma to the eye



Disease Associated with Naegleria fowleri



Identification of Amebae from Graphic Images

FLAGELLATES

GENERAL CHARACTERISTICS

1. Motility by flagella
2. Infective stage - cyst (*usually ingested in contaminated food/water*) except when no cyst stage, then troph
3. Diagnostic stage - demonstrate cysts or trophs in feces

Dientamoeba fragilis

1. Studies indicate this is actually a flagellate and not an ameba as the name might suggest
2. No cyst stage is known; pathogenic
3. Troph
   a. Usually has 2 nuclei
   b. Nucleus - has a blot-like karyosome composed of 4-8 granules
   c. Lacks flagella

TEST Alert!
D. fragilis Has No Known Cyst Stage

Giardia lamblia

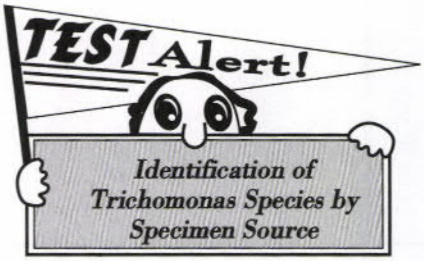
1. Pathogenic; causes duodenitis and malabsorption of fats
2. Cyst
   a. 4 nuclei
   b. Retracted flagella, axostyle and parabasal body
   c. Ovoid shaped
   d. Size 12 µm
3. Troph
   a. "Falling leaf" motility
   b. 2 nuclei
   c. Axostyle and parabasal body
   d. 4 pairs of flagella
   e. Sucker on underside of organism attaches to mucosa
   f. Tear-drop shaped
   g. Size 12 µm
4. Common in day-care centers and AIDS patients

Chilomastix mesnili

1. Nonpathogenic
2. Cyst
   a. 1 nucleus
   b. Lemon shaped
   c. Size 10 µm
3. Troph
   a. 1 nucleus
   b. Spiral groove - which gives it a "cork screw-type" motility
   c. Cytostome
   d. 4 flagella
   e. Tear-drop shaped
   f. Size 15 µm

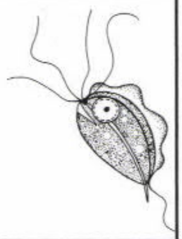
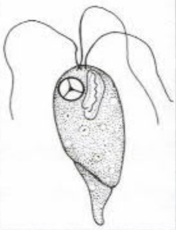
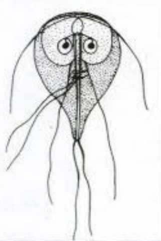
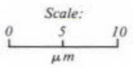
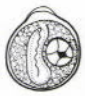
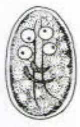
Trichomonas sp.

1. *T. vaginalis* - pathogenic; others - nonpathogenic
2. No cyst stage
3. Species can be identified by source
   a. *T.* vaginalis - vagina or urethral discharge
   b. *T. hominis* - feces


TEST Alert!
Identification of Trichomonas Species by Specimen Source

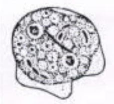
4. Troph
   a. Tear drop shaped
   b. Undulating membrane attached to flagella; length of membrane used in differentiation
      - ❖ *T. vaginalis - 1/2 length of organism*
      - ❖ *T. hominis - full length of organism*

Intestinal Flagellates: Trophs and Cysts

	Trichomonas hominis	Chilomastix mesnili	Giardia lamblia
Trophozoite			
Cyst	No cyst  		

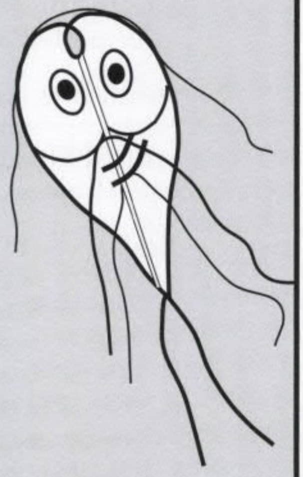
Brooke MM, Melvin DM. *Morphology of Diagnostic Stages of Intestinal Parasites,* 2nd ed., CDC, 1984

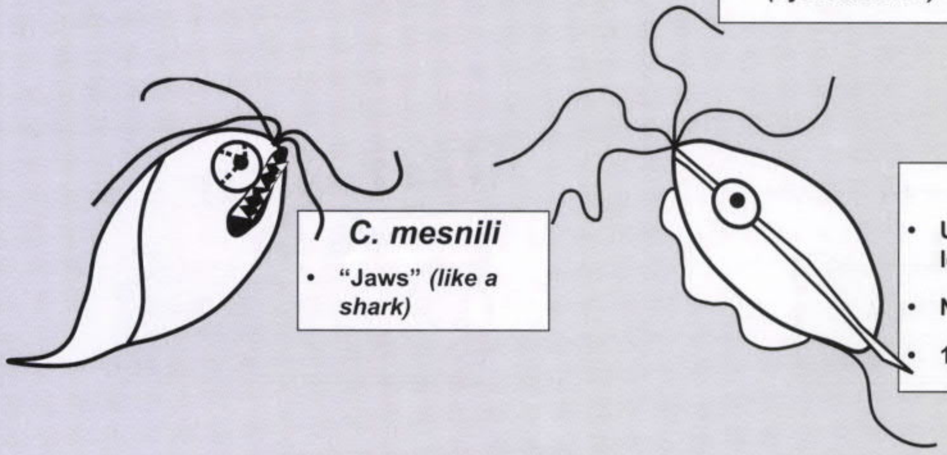
Characteristics of Dientamoeba fragilis

	Dientamoeba fragilis*
Trophozoite	
Cyst	No cyst

* Flagellate

REMEMBER! Flagellate Trophs





G. lamblia

- **"Little old lady/man" with glasses**
- **Troph too cute to make you sick *(cyst-infective)***

C. mesnili

- **"Jaws" *(like a shark)***

T. hominis

- **Undulating membrane - full length of body**
- **No cyst stage**
- **1 posterior & 4 anterior flagella**

CILIATES

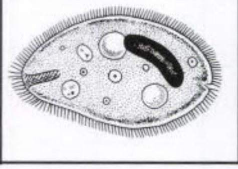
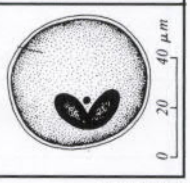
GENERAL CHARACTERISTICS

1. Motility by cilia
2. Infective stage - cyst *(ingestion of contaminated food/water)*
3. Diagnostic stage - cysts or trophs in feces

Balantidium coli

1. Largest protozoan to infect man
2. Pathogenic
3. Cyst
   a. 2 nuclei
   b. Shape - round
   c. Double cell wall
   d. Size 50 µm
4. Troph
   a. Completely covered with cilia
   b. 2 nuclei *(one large kidney bean shaped macronucleus and one small round micronucleus)*
   c. Shape - oval
   d. Size 100 µm

Sole Ciliate: Balantidium coli Troph and Cyst

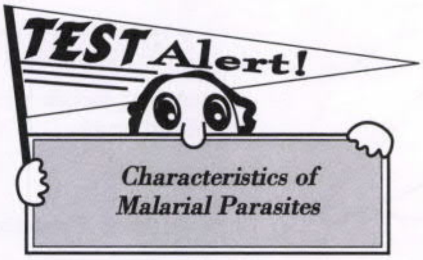
	Trophozoite	Cyst
Balantidium coli		

Brooke MM, Melvin DM. *Morphology of Diagnostic Stages of Intestinal Parasites*, 2nd ed., CDC, 1984

Plasmodium sp.

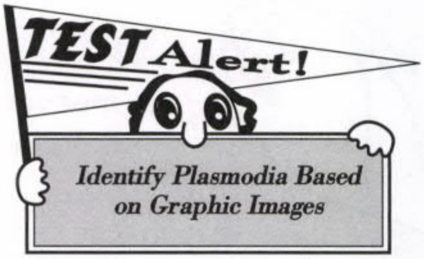
GENERAL CHARACTERISTICS

1. **Causes malaria with bouts of fever and chills**
2. **Infective stage - sporozoites injected into the bloodstream via bite of an infected female Anopheles mosquito**
3. **Diagnostic stage - demonstration of characteristic parasite in Geimsa-stained blood smears *(thick and thin)*; collect blood prior to fever spike**
4. **Resistance to infection**
   a. **Duffy negative red cells (*Fy4Fy4*) are resistant to *P. vivax* invasion**
   b. **HgbS resistance to invasion by *P. falciparum***
   c. **G-6-PD deficient cells are more resistant to invasion by *P. falciparum***



Babesia

1. ***B. microti* causes most infections in U.S.**
2. **Blood parasite of domestic animals**
3. **Infective stage - sporozoites injected via the bite of a tick**
4. **Confused with ring forms of *P. falciparum*, but lack malarial pigment**
5. **Tiny ring forms are in packets of four called "Maltese cross"**
6. **Can be transmitted via blood transfusion**
7. **Diagnostic stage - demonstration of characteristic ring forms in Giemsa stained blood smears (*thick and thin)***

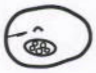
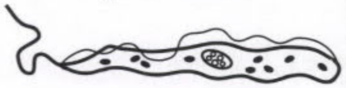

TEST Alert!
Identify Plasmodia Based on Graphic Images

Blood and Tissue Protozoa

Characteristics of Plasmodia

	P. vivax	*P. ovale*	*P. falciparum*	*P. malariae*
Appearance of Red Cell	Enlarged	Enlarged	Normal	Normal
RBC Inclusions	Schuffner's dots	Schuffner's dots	Maurer's Dots	Zieman's Stippling
Trophozoite	Ameboid	Red Cell Containing Troph May Have Fimbriated Edges	Accole´ or Applique´ Forms (Troph at Margin of Red Cell); May Have Multiple Rings	Band across cell
Average Number of Merozoites in Schizont	16	8	24	8
Stages Seen in Peripheral Blood	All	All	Few Rings and Gametocytes (Crescent or Banana-Shaped)	Young Rings, Mostly Trophs and Schizonts
Age of Infected Red Cells	Young	Young	Old	All ages
Disease	Benign Tertian	Tertian Malaria	Malignant Tertian Malaria, Blackwater Fever (Release of Hemoglobin into Urine)	Quartan Malaria

Trypanosoma and Leishmania: Stages Seen Most Often

STAGE	DESCRIPTION	COMMON NAME:	FOUND IN:
Amastigote	No Flagella	Leishmanial Form 	L. donovani L. tropica L. braziliensis T. cruzi
Trypomastigote	Flagella Originates at Posterior End of Organism	Trypanosomal Form 	T. rhodesiense T. gambiense T. cruzi

GENERAL INFORMATION: TRYPANOSOMES

1. **Hemoflagellates living in human blood and tissues**
2. **Clinical disease**
   - a. **Chagas' Disease - *T. cruzi***
   - b. **West African sleeping sickness - *T. gambiense***
   - c. **East African sleeping sickness - *T. rhodesiense***

Trypanosoma rhodesiense and gambiense

1. **Infective stage - trypomastigote injected when Tse Tse fly takes a blood meal**
2. **Diagnostic stage - demonstration of trypomastigote from Giemsa stained thick and thin smears; detected in aspirates of the chancre at the site of the insect bite, enlarged lymph node, spinal fluid or human blood**

Trypanosoma cruzi

1. **Infective stage - trypomastigote injected when feces of reduvid bug are rubbed into bite wound when host scratches the bite**
2. **Diagnostic stage**
   - a. **Demonstration of trypomastigote from Giemsa stained thick and thin smears as well as aspirate from chancre at the site of the insect bite, enlarged lymph node, and spinal fluid**
3. **Can be transmitted by blood transfusion**

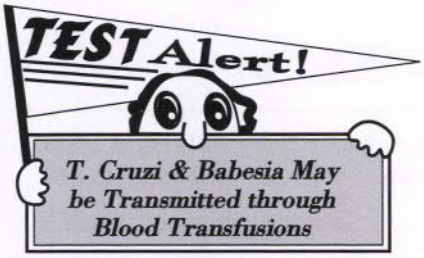
Characteristics of Trypanosomes

	T. cruzi	*T. gambiense*	*T. rhodesiense*
Diagnostic Stage	Amastigote (Striated Muscle-Heart and GI Tract) Trypomastigote (Blood)	Trypomastigote (Blood)	Trypomastigote (Blood)
Characteristics:	Trypomastigote - "C" or "U" shape	Long and Slender with Undulating Membrane	Long and Slender with Undulating Membrane
Type of Smear	Blood Smear: Giemsa-Stained Thick/Thin Aspirate: Chancre, Lymph Node or CSF	Blood Smear: Giemsa-Stained Thick/Thin Aspirate: Chancre, Lymph Node or CSF	Blood Smear: Giemsa-Stained Thick/Thin Aspirate: Chancre, Lymph Node or CSF
Human Infected By:	Feces from Reduvid Bug (Kissing Bug) Rubbed into Bite Wound; Blood Transfusions and Placental Transfer	Bite of Tse Tse Fly	Bite of Tse Tse Fly
Disease	Chagas' Disease South American Sleeping Sickness	West African Sleeping Sickness	East African Sleeping Sickness

REMEMBER!

T. cruzi

Cruzi begins with "C" and so does Chagas, and trypomastigote seen in characteristic "C" shape



T. Cruzi & Babesia May be Transmitted through Blood Transfusions

GENERAL INFORMATION: LEISHMANIA

1. Clinical disease
   a. *L. donovani* - causes Visceral Leishmaniasis or Kala Azar
   b. *L. tropica* - causes Old World Cutaneous Leishmaniasis or Oriental sore
   c. *L. braziliensis* - causes New World Cutaneous or Mucocutaneous Leishmaniasis

2. Infective stage - promastigotes released via bite of a sandfly

3. Diagnostic stage - demonstration of amastigote in macrophage in Giemsa stained smears from aspirates or biopsies of skin lesions

INTESTINAL COCCIDIA

GENERAL CHARACTERISTICS

1. Prevalent in AIDS patients / immunocompromised persons
2. Diagnostic stage: oöcysts passed in feces
3. Infective stage: oöcysts

Cryptosporidium parvum

1. Method of infection
   a. Infective oöcysts transmitted via fecal-oral route

2. Clinical disease
   a. Illness is self-limiting in immunosufficient persons
   b. Immunodeficient persons experience severe diarrhea, fever, nausea, abdominal pain and weight loss

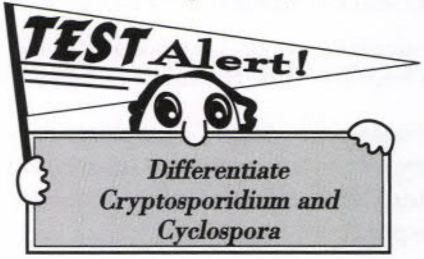
3. Diagnosis
   a. Modified acid-fast stain
      - ❖ *Oöcysts stain red against blue background (acid fast positive)*
      - ❖ *Average size is 4-6 µm*

Cyclospora cayetanensis

1. Method of infection
   a. Infective oöcysts ingested in contaminated food or water
   b. Outbreaks have been associated with contaminated produce (berries, lettuce, etc); usually during spring & summer months

2. Clinical disease - indistinguishable from cryptosporidiosis

3. Diagnosis
   a. Modified acid-fast stain
      - ❖ *Oöcysts stain from light pink to deep red (acid-fast variable)*
      - ❖ *Average size is 8-10 µm (larger than C. parvum)*



Differentiate Cryptosporidium and Cyclospora

Isospora belli

1. Method of infection
   a. Direct transmission to humans via fecally contaminated food or water

2. Clinical disease
   a. Often asymptomatic and self-limiting
   b. Symptoms range from mild gastro-intestinal distress to severe dysentery

3. Diagnosis
   a. Modified acid-fast stain
      - ❖ *Sporoblasts and/or sporocysts stain deep red (acid-fast positive)*

Microsporidia

1. Newest group of obligate intracellular organisms; includes 7 genera closely related to the zygomycetes (fungi)

2. *Enterocytozoon bieneusi* - most common microsporidia causing enteritis in AIDS patients

3. Organism is very small *(1.5 - 4 µm)*

4. Characteristic feature - spores containing a polar tubule, used to inject infective spore contents into host

5. Method of infection
   a. Most likely by ingestion of spores
   b. Inhalation of spores, ocular exposure and sexual intercourse may also be routes of transmission

6. Clinical disease
   a. Similar to cryptosporidiosis
   b. Spores are very resistant

7. Diagnosis
   a. Modified Trichrome stain
      ❖ *Concentration 10 x higher than traditional trichrome stain*
      ❖ *Spore wall stains bright pink; background stains green or blue (depending on counterstain)*
   b. Serological testing
   c. PCR - research labs only
   d. Electron microscopy - necessary to speciate; not available in most clinical laboratories

TISSUE COCCIDIA

GENERAL CHARACTERISTICS

1. Prevalent in AIDS patients / immunocompromised persons

2. Worldwide distribution

Toxoplasma gondii

1. Method of infection
   a. Definitive host: house cat
   b. Accidental ingestion / inhalation of oöcysts from cat feces (*when cleaning litter box)*
   c. Ingestion of undercooked meat (*cattle, sheep, pigs*) - intermed. hosts
   d. Transmitted across the placenta to fetus from mother who acquires first infection during pregnancy

2. Clinical disease
   a. Infection in immunosufficient persons usually asymptomatic
   b. Symptoms resemble infectious mononucleosis
   c. Can cause death of fetus, mental retardation or blindness when transmitted across placenta

3. Diagnosis
   a. Serological testing (*EIA, IFA*); important in detecting neonatal toxoplasmosis (*IgM antibodies*)

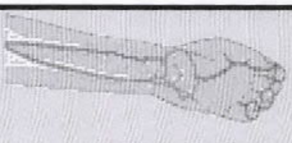
Pneumocystis jiroveci (previously P. carinii)

1. Recently classified as a fungus, based on DNA studies

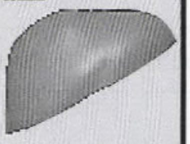
2. Causative agent of atypical interstitial plasma-cell pneumonia (*PCP*)

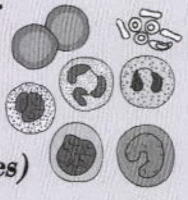
3. Identified with Gomori's methenamine silver stain (*silver impregnation stain*)

REMEMBER!
Beyond Stools:
Look for me in:



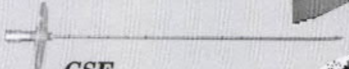
LIVER / SPLEEN
Entamoeba histolytica
Leishmania donovani



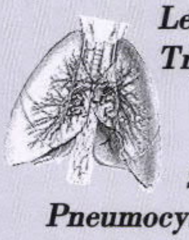


BLOOD
Plasmodium (red cells)
Babesia (red cells)
Leishmania (macrophages)
Trypanosoma (plasma)

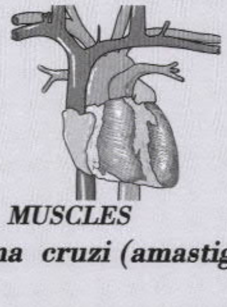
SKIN
Leishmania



CSF
Naegleria fowleri



SPUTUM
Pneumocystis jiroveci
Cryptosporidium parvum



MUSCLES
Trypanosoma cruzi (amastigote)

EYE
Acanthamoeba

URINE
Trichomonas vaginalis

Some Diseases Caused by Human Parasites

DISEASE	PARASITE
Amebiasis	*Entamoeba histolytica*
Anemia	Hookworms
Blindness	*Onchocerca volvulus*
Chagas Disease	*Trypanosoma cruzi*
Cysticercosis	*Tania solium*
Cutaneous Larval Migrans	Dog and cat hookworms (*Ancylostoma brazilliensis)*
Visceral Larval Migrans	Dog and cat ascarids (*Toxocara canis and cati*)
Dum Dum Fever (kala-azar)	*Leishmania donovani*
Elephantiasis	*Wucheria bancrofti, Brugia malayi*
Hydatid Disease	*Echinococcus granulosus*
Malaria	*Plasmodium species*
Meningoencephalitis	*Naegleria fowleri*
Oriental Sore	*Leishmania tropica*
Sleeping Sickness, East African	*Trypanosoma rhodesiense*
Sleeping Sickness, West African	*Trypanosoma gambiense*
Sparganosis	*Diphyllobothrium latum*
Trichinosis	*Trichinella spiralis*
Toxoplasmosis	*Toxoplasma gondii*

Vectors & Insects Transmitting Parasites

VECTORS AND INSECTS	PARASITES
MOSQUITOES Anopheles	*Malaria, Wucheria bancrofti, B. malayi*
Culex	*Wucheria bancrofti*
Mansonia	*B. malayi*
FLIES Simulium (black fly)	*Onchocerca volvulus*
Chrysops fly	*Loa Loa*
Sandfly	*Leishmania*
Tse Tse fly	*T. gambiense, T. rhodesiense*
FLEAS Dog flea	*Dipylidium caninum*
Rat flea	*Hymenolepis diminuta*
Copepods (water flea)	*D. latum*
TICKS Deer tick	*Babesia*
BUGS Reduvid bug	*T. cruzi*

MISCELLANEOUS TESTS

1. Occult blood - "Hidden Blood"
   - a. Used for early detection of colorectal cancer
   - b. Principle - pseudoperoxidase activity of hemoglobin releases oxygen from hydrogen peroxide to oxidize guaiac reagent
   - c. Interpretation - blue color indicates gastrointestinal bleeding
2. Fecal fat
   - a. Steatorrhea - indicated when fecal fat excretion > 6g/day
   - b. Stain with Sudan III, Sudan IV, or Oil Red O
   - c. Characteristic orange-red staining

PARASITOLOGY SAMPLE QUESTIONS

1. Which of the following organisms causes Visceral Larval Migrans?
   A. ***Ancyclostoma brazilensis***
   B. ***Ascaris lumbricoides***
   C. ***Strongyloides stercoralis***
   D. ***Toxocara canis***

2. Enterobius vermicularis infection is usually diagnosed by finding eggs from
   A. **Cellulose tape preps.**
   B. **Concentrated stool samples.**
   C. **Iodine wet mounts from fresh stool.**
   D. **Sedimented stool samples.**

3. The infective stage for *Strongyloides stercoralis* is the
   A. **Ova.**
   B. **Filariform larvae.**
   C. **Rhabditiform larvae.**
   D. **Free living adult.**

4. If humans ingest the egg of *Taenia solium*, they may develop
   A. **Hydatid Disease.**
   B. **Sparganosis.**
   C. **Trichinosis.**
   D. **Cystercercosis.**

5. A silver methenamine stained slide of a bronchial washing from an AIDS patient is examined. Which of the following organisms is most likely to be identified?
   A. ***Cryptosporidium parvum***
   B. ***Toxoplasma gondii***
   C. ***Strongyloides stercoralis***
   D. ***Pneumocystis jiroveci***

6. A blood smear showed crescent shaped gametocytes in several red cells. What is the most probable identity of the organisms?
   A. ***Babesia microti***
   B. ***Plasmodium falciparum***
   C. ***Plasmodium malariae***
   D. ***Leishmania donovani***

7. Which organism is transmitted to humans by ticks and blood transfusions?
   A. ***Babesia***
   B. ***Wuchereria bancrofti***
   C. ***Leishmania donovani***
   D. ***Trypanosoma cruzi***

8. The ameba which can cause liver or lung abscesses is
   A. ***Balantidium coli***
   B. ***Dientamoeba fragilis***
   C. ***Endolimax nana***
   D. ***Entamoeba histolytica***

9. Which of the following can cause a fatal meningoencephalitis?
   A. ***Balantidium coli***
   B. ***Entamoeba histolytica***
   C. ***Naegleria fowleri***
   D. ***Toxoplasmosis gondii***

10. The trophozoite of which organism is characterized by "falling leaf motility", two nuclei, an axostyle, 4 pair of flagella and a sucker on the underside of the organism?
    A. ***Chilomastix mesinili***
    B. ***Giardia lamblia***
    C. ***Trichomonas hominis***
    D. ***Trichomonas vaginalis***

11. Which of the following eggs may not be detected in zinc flotation procedures?
    A. ***Ascaris lumbricoides*** **(fertile)**
    B. ***Trichiura trichuris***
    C. ***Schistosoma mansoni***
    D. ***Taenia solium***

12. Which trophozoite is suspected if ingested red cells are seen on a saline wet prep?
    A. ***Entamoeba coli***
    B. ***Entamoeba histolytica***
    C. ***Endolimax nana***
    D. ***Iodamoeba butschlii***

13. Pregnant women are to avoid cleaning litter boxes of their house cats until after delivery to prevent congenital infection of
    A. ***Ancylostoma caninum***
    B. ***Dipylidium caninum***
    C. ***Toxocara cati***
    D. ***Toxoplasma gondii***

14. A patient visiting from overseas is hospitalized due to suspected tuberculosis. The sputum sample submitted to the laboratory is bloody with orange-brown flecks. Preliminary TB tests are negative. Which of the following parasites may be suspected?
    A. ***Ascaris lumbricoides***
    B. ***Fasciolopsis buski***
    C. ***Schistosoma japonicum***
    D. ***Paragonimus westermani***

15. Which of the following cysts has a nucleus with a prominent karyosome, a clear halo, no achromatic granules, no peripheral chromatin?
    A. ***Entamoeba histolytica***
    B. ***Entamoeba hartmanni***
    C. ***Endolimax nana***
    D. ***Iodamoeba bütschlii***

16. Differential diagnosis of *Cryptosporidium parvum* and *Cyclospora cayetanensis* is based on:
    - A. Clinical symptoms
    - B. Oöcyst size - Cyclospora is larger
    - C. Oöcyst morphology
    - D. Acid fast stained smears - Cyclospora is NOT acid fast

ANSWERS AND RATIONALE

1. D

Dog & Cat Ascarids *(Toxocara canis and cati)* cause Visceral Larval Migrans. Option A (*Dog and Cat Hookworms*) causes Cutaneous Larval Migrans. Option B may cause pneumonia from migration through liver and lungs or bowel obstruction. Option C may cause abdominal pain, vomiting and diarrhea.

2. A

The female migrates to the anal opening *(usually at night)* and lays eggs in the perianal opening. The cellulose tape (*scotch tape*) prep will pick up the eggs and when stuck to a slide will allow microscopic viewing.

3. B

The filariform larvae produces a proteolytic enzyme which allows it to penetrate man's skin.

4. D

Taenia solium causes cystercercosis. Option A results from an infection with Echinococcus granulosus. Option B occurs when man is the intermediate host of Diphyllobothrium latum. Option C occurs when the larval forms of Trichinella spiralis encysts in the muscle.

5. D

Pneumocystis jiroveci causes interstitial plasma cell pneumonia and is life threatening to immunocompromised patients. Option A can cause profuse diarrhea in AIDS patients and is identified using an acid fast stain. Option B leads to involve the CNS with various neurological symptoms and is diagnosed using serologic techniques. Option C may cause death and has a high rate of autoinfection in immunocompromised hosts but is diagnosed by characteristic ova or larvae in stool samples.

6. B

The crescent shaped gametocytes is characteristic of *P. falciparum.*

7. A

There is documentation of Babesia transmission via blood transfusion as well as deer ticks. The other three options are not transmitted by a tick bite (*Wucheria* - mosquito, *Leishmania* - sandfly, *T. cruzi* - reduvid bug).

8. D

A hallmark of *E. histolytica* is its ability to form extraintestinal abscesses. Options A and B are not amebas and do not cause extraintestinal abcesses. Option C is an ameba but is nonpathogenic.

9. C

Naegleria fowleri can cause primary amebic meningoencephalitis; infections may go undetected unless a direct wet prep of the CSF is performed.

10. B

Other choices have one nuclei and no sucker.

11. C

The eggs of *S. mansoni* are too large and heavy to float. Other eggs which may not be demonstrated in the zinc flotation procedure are infertile Ascaris and those with operculi such as *Diphyllobothrium latum*

12. B

The diagnostic feature of *E. histolytica* is the finding of ingested red cells in trophs. Options A, C and D may exhibit ingested bacteria.

13. D

The definitive host of *T. gondii* is the cat. Immunocompromised patients as well as pregnant women are advised to avoid cleaning litter boxes to prevent exposure. Most people exhibit few or no symptoms due to infections, but immunocompromised patients may experience cyst rupture leading to internal lesions while pregnant women may pass the infection transplacentally leading to congenital problems.

14. D

P. westermani, a lung trematode found in the far East and Africa, encapsulates in the lungs. The brownish-yellow eggs may be found in sputum or fecal samples. Eggs of options A, B and C are found in the stool.

15. C

Options A and B exhibit peripheral chromatin. Option D has achromatic granules.

16. B

Cyclospora is about twice the size of *Cryptosporidium.* Both organisms are acid fast *(Cyclospora is variable*) and have similar morphology and clinical symptoms.

☞ Be able to recognize the more common parasite eggs, cysts or trophozoites from graphic images.

MOLECULAR DIAGNOSTICS & GENETICS

by Mona Bakeer, Elizabeth Williams, Marcia Firmani

Key Terminology

Agarose – a gel-forming polysaccharide extracted from seaweed

Allele – alternative forms of a gene that is present at a given locus

Cell nucleus – carries genetic information

Codon – 3 bases in mRNA that code for amino acid production

Diploid - cells that carry two genome copies

Epigenetics - study of changes in the regulations of gene activity and expression that are not dependent on gene DNA sequence

Exon - coding DNA (*translated into a protein*)

Gene Expression - protein synthesis (*gene product*)- is tightly controlled and regulated

Genetic Code – combination of nucleotides that build the different codons

Genome - an organism's total DNA content

Genotype – the observed alleles for an individual at a genetic locus

Haploid- -cells that contain a single copy of the genome such as germ cells or gametes

Haplotype – series of alleles on a single chromosome

Heterozygous – two different alleles at a locus

Homozygous – two identical alleles at a locus

Intron- non-coding DNA between 2 exons

Ligation – process of joining two DNA molecule ends. It involves creating a phosphodiester bond between 3' hydroxy of one nucleotide and the 5'phosphate of another.

Linkage disequilibrium – allelic association when closely linked alleles are inherited together during many generations

Locus – location of a gene in the genome

Molecular Diagnostics – the use of DNA, RNA or mRNA to identify and /or characterize disease caused by infectious agents or gene abnormalities

Mutations - changes in the DNA sequence

Penetrance - the probability of expressing a phenotype, given a particular genotype

Phenotype – observable characteristics

Polymorphism – a variation in the base sequence of DNA

Proteins – made up of amino acids

Protein expression - Different proteins are expressed in different cells according to the function of the cell

Proteomics - organism's complete complement of proteins

RFLP (***Restriction Fragment Length Polymorphism***)- variation in the size of DNA fragments generated by restriction enzymes

Sequence - the order of nucleotide bases along a DNA strand

STR (*short tandem repeats*) - Short sequences of DNA, normally 2-5 base pairs, that are repeated numerous times

Telomere - Region of repetitive DNA at the end of a chromosome

Transcription – process where genetic information in DNA is copied into messenger RNA (*mRNA*) utilizing the RNA polymerase enzyme

Transcriptome - set of all RNA molecules transcribed in a cell

Transgene - a foreign gene that is introduced randomly somewhere in the genome

Translation – making a protein using the information provided by mRNA

VNTR (*variable number of tandem repeats*) - Repeats of identical nucleotide sequences lined up one after another that vary in number from one individual to another i.e., gaca gaca gaca gaca gaca gaca

Molecular Diagnostics

APPLICATION

1. Detect cause of disease state (*diagnosis*)
2. Predict disease progression
3. Paternity and forensic analysis
4. Gene therapy and drug design

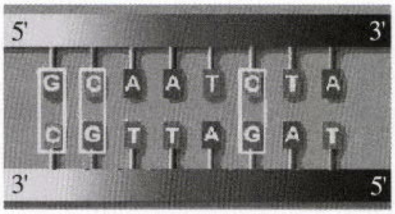
Genetics and Molecular Biology

DNA THE CHEMICAL BASIS OF HEREDITY

1. Biological "blueprint"
2. Carries information for cells to live, grow, differentiate, and replicate
3. Provides consistency and variability

GENETIC UNITS OF HUMAN DNA

1. Nuclear DNA
    a. Diploid genome *(two sets of chromosomes)*
    b. Packaged in 23 pairs of chromosomes
    c. 22 homologous pairs *(autosomes)*
    d. 2 sex chromosomes (*XX or XY*)
    e. 6 billion bases
    f. Approximately 30,000 genes
2. Mitochondrial DNA (*non-nuclear*)
    a. 16,569 base pairs
    b. 37 genes
    c. Higher mutation rate
    d. 128 naturally occurring polymorphisms
    e. Maternal inheritance
3. DNA/ RNA comprised of 2 types of nitrogen bases:
    a. Purines
        - *Adenine A DNA RNA*
        - *Guanine G DNA RNA*
    b. Pyrimidines
        - *Cytosine C DNA RNA*
        - *Thymine T DNA*
        - *Uracil U RNA*
    d. Hydrogen bonds can form between a pyrimidine and a purine
    e. Watson-Crick base pairing $A=T, G\equiv C$
4. DNA double helix
    a. Nucleotide bases pair together to form "base pairs", A always binds to T (*U in RNA*), C always binds to G
    b. DNA double helix are oriented in opposite directions.
    c. 5' end is beginning of DNA strand
    d. 3' end is end of DNA strand

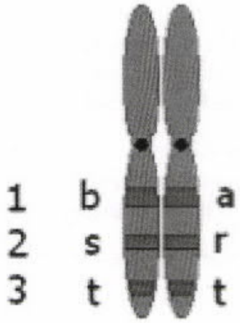


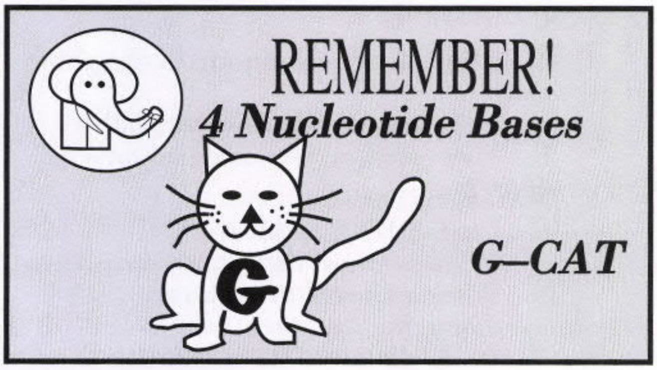
DNA REPLICATION (*SEMI-CONSERVATIVE*)

1. DNA strands separate (*helicase enzyme*)
2. Pairing the bases in each strand with new bases to form complementary strands (*DNA polymerase)*
3. Produce two new DNA strands (*exact duplicate of original DNA*)

THE HUMAN CHROMOSOME

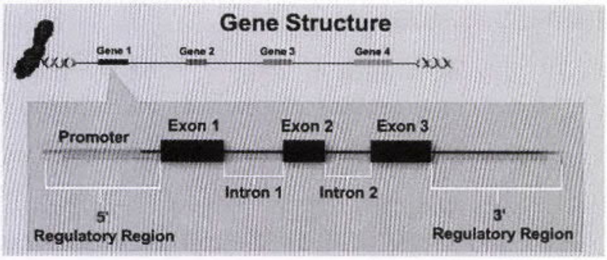
1. Single linear duplex DNA
2. Numerous protein interactions
3. Karyotype
    a. Looking for normality in number and structure
    b. Numbered by size and centromere position
        - *short arm -p*
        - *long arm - q*
        - *regions counted from centromere out*
    c. Pattern produced by intensity of stain or fluoresence
    d. Genotype – genes on homologous chromosomes
        - *gene 1 – a,b*
        - *gene 2 – r,s*
        - *gene 3 – t,t*
    e. Haplotype – genes on one chromosome
        - *left chromosome – at a particular locus defining genes 123 – b,s,t*
        - *right chromosome – genes 123- a,r,t*




REMEMBER!
4 Nucleotide Bases
G
G–CAT

HUMAN GENE STRUCTURE

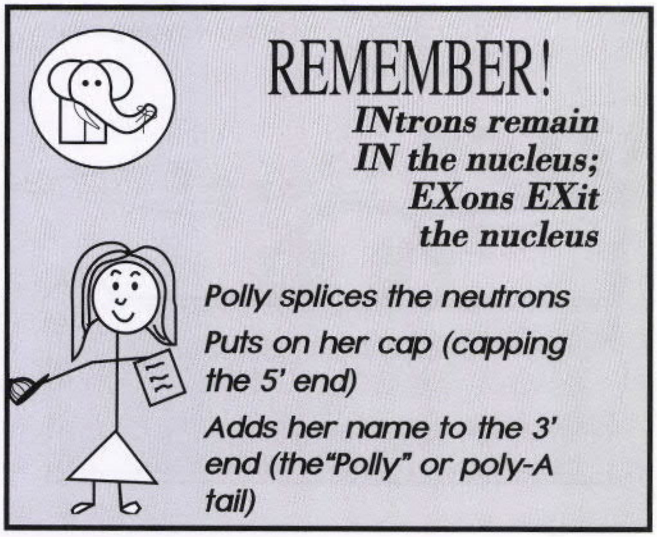
1. Genes
    a. Exons separated by introns
    b. Sequence of base pairs encodes information for proteins.
    c. Different sequence = different protein

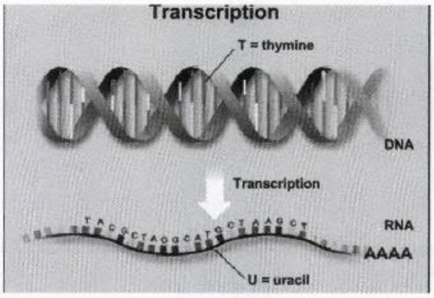

Gene Structure
Gene 1
Gene 2
Gene 3
Gene 4
Promoter
Exon 1
Exon 2
Exon 3
Intron 1
Intron 2
5'
Regulatory Region
3'
Regulatory Region

TRANSCRIPTION

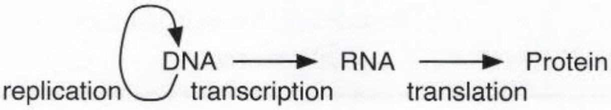
1. mRNA sequence - complementary to the DNA template
    a. Uracil (*U*) bases replace thymine (*T*) bases in RNA

2. mRNA processed by transcription:
    a. Splicing – removal of introns
    b. Capping – modify the 5' end
    c. Polyadenylations – add adenines to the 3 'end (*also called poly-A tail*)


REMEMBER!
INtrons remain IN the nucleus; EXons EXit the nucleus
Polly splices the neutrons
Puts on her cap (capping the 5' end)
Adds her name to the 3' end (the"Polly" or poly-A tail)


Transcription
T = thymine
DNA
Transcription
RNA
AAAA
U = uracil

The Central Dogma of Molecular Biology


DNA
RNA
Protein
replication
transcription
translation

TRANSLATION / GENETIC CODE

1. Each combination of 3 nucleotides on mRNA is called codon

2. Each codon specifies a particular amino acid

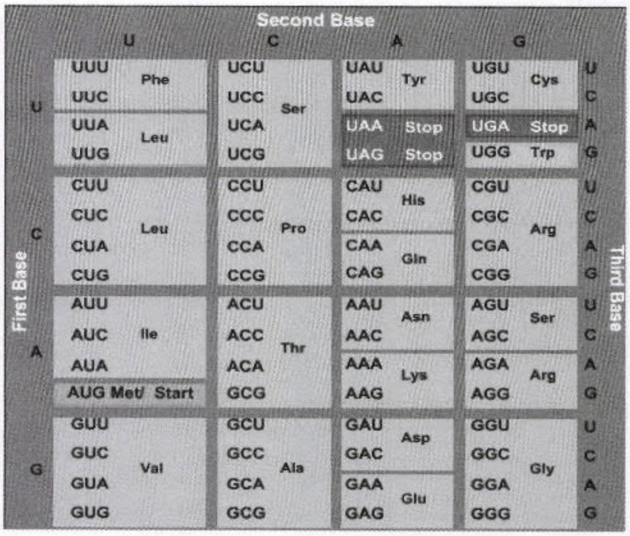
3. Each amino acid is to be placed in the polypeptide chain (*protein*)

mRNA
5´...NNNNNNN**AUG**CUCGGGAGCCAUUAANNNNN...3´
Translation Start Site
Translation Stop Signal

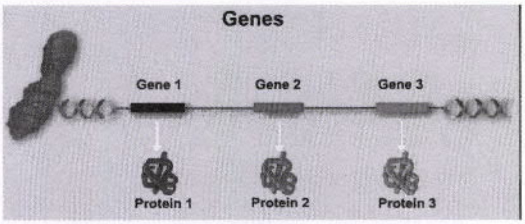
Codon Usage AUG-CUC-GGG-AGC-CAU-UAA

Translation

Peptide Sequence Met-Leu-Gly-Ser-His



First Base	Second Base U		Second Base C		Second Base A		Second Base G		Third Base
U	UUU	Phe	UCU	Ser	UAU	Tyr	UGU	Cys	U
	UUC		UCC		UAC		UGC		C
	UUA	Leu	UCA		UAA	Stop	UGA	Stop	A
	UUG		UCG		UAG	Stop	UGG	Trp	G
C	CUU	Leu	CCU	Pro	CAU	His	CGU	Arg	U
	CUC		CCC		CAC		CGC		C
	CUA		CCA		CAA	Gln	CGA		A
	CUG		CCG		CAG		CGG		G
A	AUU	Ile	ACU	Thr	AAU	Asn	AGU	Ser	U
	AUC		ACC		AAC		AGC		C
	AUA		ACA		AAA	Lys	AGA	Arg	A
	AUG	Met/ Start	GCG		AAG		AGG		G
G	GUU	Val	GCU	Ala	GAU	Asp	GGU	Gly	U
	GUC		GCC		GAC		GGC		C
	GUA		GCA		GAA	Glu	GGA		A
	GUG		GCG		GAG		GGG		G



REMEMBER!

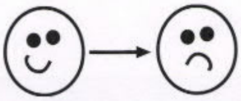
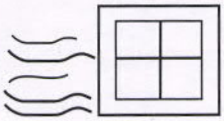
Order of Nucleotides = Amino Acid

Order of Amino Acids = Protein

GENETIC MUTATIONS

1. Nucleotide sequence alterations
   a. Point mutations
   b. Insertions
   c. Deletions
2. Large scale alterations
   a. Chromosomal rearrangements
   b. Chromosomal deletions
   c. Gene amplification

SPECIFIC TYPES OF MUTATIONS

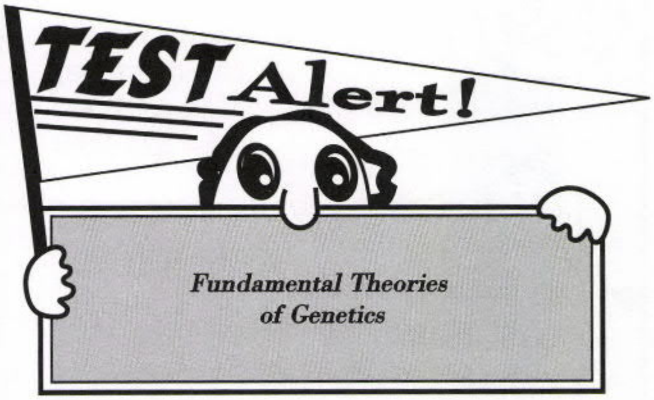
	MUTATION TYPE	WHAT HAPPENS
	Germ-Line	Can be inherited and transmitted
	Missense	Nucleotide in a sequence is altered such that the amino acid changes
	Silent	Nucleotide in a sequence is altered such that the amino acid does not change
	Nonsense	Nucleotide changes such that a stop codon is introduced
	Frameshift	Nucleotide is either deleted or added to a sequence → the protein produced is altered

SOMATIC MUTATIONS

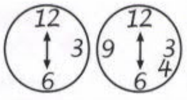
1. Mutations can accumulate in daughter cells
   a. Cells may divide uncontrollably
   b. May result in tumor development
2. Mutations occur:
   a. During DNA replication
      ❖ *Incorporation of the wrong bases*
   b. When exposed to mutagens *(DNA damaging agents)*
      ❖ *Radiation, UV, x-rays, radioactivity*
      ❖ *Chemicals that bind to or react with DNA*
      ❖ *Reactive oxygen compounds damage DNA*
   c. Polymorphism if relative frequency is >1%

GENETIC DISEASES

1. Chromosomal defects
   a. Gain or loss of a whole chromosome (*aneuploidy*)
   b. Gain or loss of part or parts of a chromosome (*deletion*)
   c. Transfer of a segment of one chromosome to another *(translocation)*
   d. Reversal of a segment of a chromosome (*inversion*)
2. Single-gene disorders
   a. Dominant or recessive
   b. X-linked or autosomal
3. Multigenic traits
   a. Difficult to analyze genetically
   b. Do not show clear inheritance patterns
   c. Environmental factors can contribute to disease development



Fundamental Theories of Genetics

TYPE OF DEFECT	DESCRIPTION	DESCRIPTION
Aneuploidy	Gain or loss of a chromosome	
Deletion	Loss of part of a chromosome	
Translocation	Transfer of a segment of one chromosome to another	
Inversion	Reversal of a segment of a chromosome	

Mendelian Genetics

1. **Genes inherited in pairs: one copy from mom and the other from dad**
2. **Autosomal traits – inherited on an autosome *(not the X or Y chromosome)***
   a. **Autosomal dominant**
      - ❖ ***A single gene is sufficient to generate a specific phenotype***
      - ❖ ***Affected individuals have an affected parent***
      - ❖ ***Each child of an affected parent has a 50% risk of inheriting the abnormal allele***
      - ❖ ***Unaffected individuals do not have affected children***
      - ❖ ***Males and females are affected equally***

Example 1:
Two carrier parents of an autosomal dominant disease (D):
Genotype

	D	d
D	DD	Dd
d	Dd	dd

1/4 (25%) = Normal dd
1/2 (50%) = Heterozygous affected Dd
1/4 (25%) = Homozygous affected DD
Phenotype
3/4 (75%) = Affected DD, Dd
1/4 (25%) = Normal dd

Example 2:
One affected parent and one normal parent of an autosomal dominant disorder (D):

	d	d
D	Dd	Dd
d	dd	dd

Genotype and Phenotype
1/2 (50%) = Normal - dd
1/2 (50%) = Affected - Dd

   b. **Autosomal recessive**
      - ❖ ***Requires two genes, one from each parent, for the recessive phenotype***
      - ❖ ***Located on an autosome***
      - ❖ ***Carriers – one dominant gene and one recessive gene – masks recessive phenotype***
      - ❖ ***Carrier parents have a 25% chance of having an affected child***
      - ❖ ***Affected individuals may have unaffected parents (disease [phenotype] skips a generation when recessive gene is not displayed by carriers)***

Example 1:
Two carrier parents of an autosomal recessive disease (r):

	R	r
R	RR	Rr
r	Rr	rr

Genotype
1/4 (25%) = Normal RR
1/2 (50%) = Carriers Rr
1/4 (25%) = Affected rr

Phenotype
3/4 (75%) = Normal RR, Rr
1/4 (25%) = Affected rr

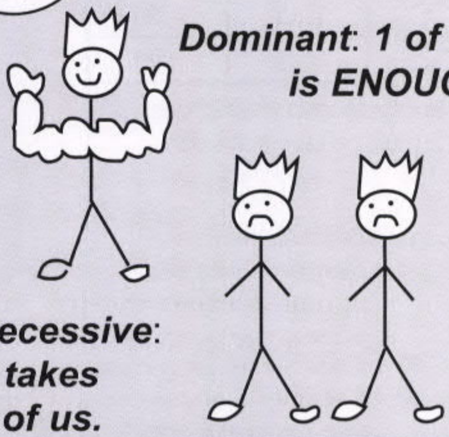
Example 2: One normal and one carrier parent of an autosomal disorder:

	R	r
R	RR	Rr
R	RR	Rr

Genotype
1/2 (50%) = Normal RR
0 = Affected
1/2 (50%) = Carriers Rr (*unaffected*)
Phenotype
All (100%) = Normal – RR, Rr

REMEMBER!
Mendelian Genetics

Dominant: 1 of me is ENOUGH.



Recessive: It takes 2 of us.

3. X-linked traits–mutations in the X-chromosome
   a. X-linked recessive
      - *Affected individuals are primarily male*
      - *All female offspring of an affected male will be carriers of the disease gene (obligate carriers)*
      - *Male offspring of affected men are not affected*
      - *Require two copies of the defective gene in females, but in males only one copy is needed as they only have one X chromosome*

Example 1:
Normal father and carrier mother:

	X_H	Y
X_H	X_HX_H	X_HY
X_h	X_HX_h	X_hY

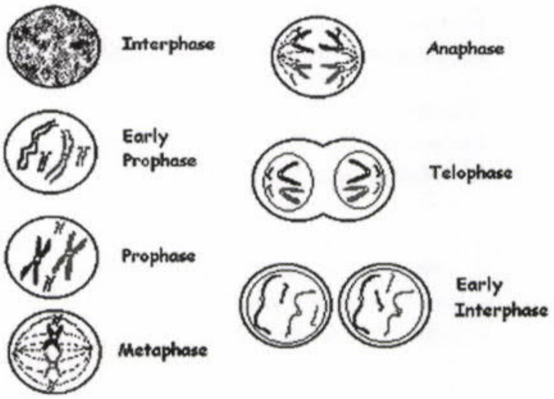
Genotype
female (50%) = Normal - XH XH
female (50% = Heterozygous Carrier - XH Xh
males (50%) - Hemizygous Normal - XHY
males (50%) - Hemizygous Affected - XhY

Phenotype
all females (100%) = Normal XH XH and XH Xh
males (50%) - Hemizygous Normal - XHY
males (50%) - Hemizygous Affected - XhY

   b. X-linked dominant
      - *All female offspring of affected males will be affected*
      - *No male offspring of affected males will be affected*
      - *An affected female has a 50% chance of having an affected child*
      - *All affected individuals have an affected parent*

MITOSIS

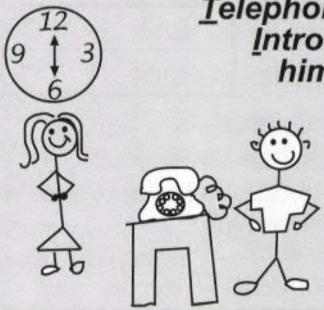
1. Cell division of somatic cells
2. A diploid cell generates an identical diploid cell
3. Normally no recombination takes place



REMEMBER!
Mitosis

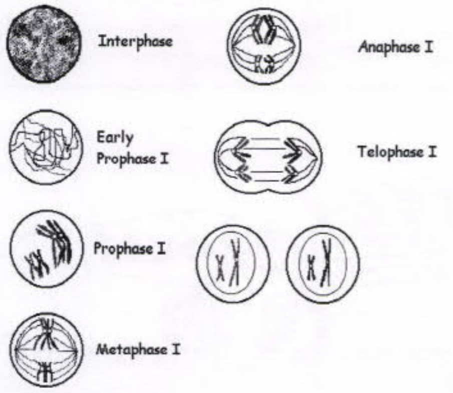
The Pro Met Ana at the Telephone to Introduce himself.

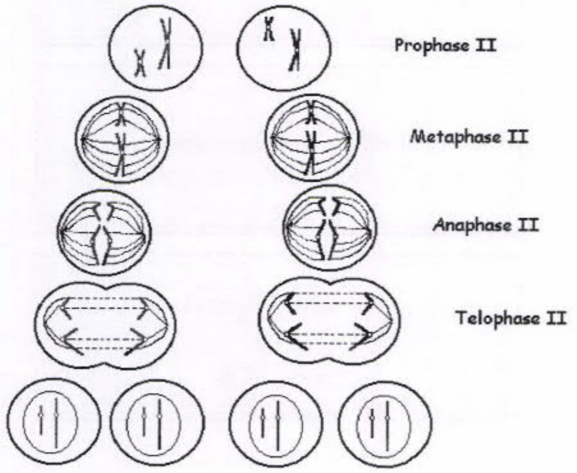
Prophase
Metaphase
Anaphase
Telophase
Interphase



MEIOSIS

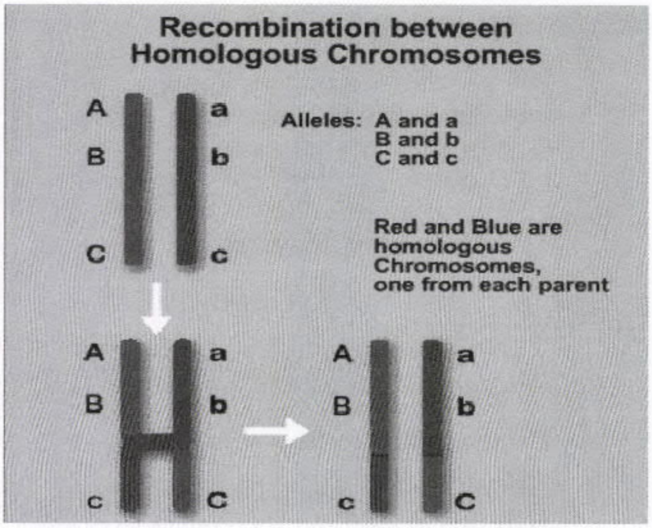
1. Occurs during gamete formation
2. Diploid progenitor cell generates four haploid gametes
3. Meiotic recombination is frequent


Interphase
Anaphase I
Early Prophase I
Telophase I
Prophase I
Metaphase I


Prophase II
Metaphase II
Anaphase II
Telophase II

CHROMOSOMAL CROSSOVER (*GENETIC RECOMBINATION*)

1. Two chromosomes (*paired up during prophase I of meiosis*) exchange some portion of their DNA
2. Matching regions of matching chromosomes break then reconnect to other chromosome (*exchange of genes*)


Recombination between Homologous Chromosomes
A
B
C
a
b
c
Alleles: A and a
B and b
C and c
Red and Blue are homologous Chromosomes, one from each parent
A
B
c
a
b
C
A
B
c
a
b
C

Molecular Techniques

BASIC STEPS IN ISOLATING DNA FROM CLINICAL SPECIMENS

Separate WBCs from RBCs, if necessary

↓

Lyse WBCs or other nucleated cells

↓

Denature / **Digest** proteins

↓

Separate contaminants (e.g., proteins, heme) from DNA

↓

Precipitate DNA, if necessary

↓

Resuspend DNA in final buffer

DNA ISOLATION METHODS

1. Liquid phase organic extraction (*phenol/chloroform*)
2. Liquid phase non-organic extraction
3. Solid phase procedures

ISOLATION AND PURIFICATION OF NUCLEIC ACID

1. Sample source:
   a. Any tissue with nucleus
   b. Blood collected in anticoagulant
      - ❖ *EDTA preferred*
      - ❖ *DO NOT FREEZE WHOLE BLOOD*
2. Storage conditions
   a. Store DNA in TE buffer at 4°C for weeks and at -20°C to -70°C– for long term
   b. Store RNA in RNase free ultrapure water at -70°C

NUCLEIC ACID ANALYSIS

1. DNA or RNA quantity, quality and molecular size characterized by
   a. UV spectrophotometry
   b. Agarose gel electrophoresis
   c. Fluorometry
   d. Colormetric blotting

NUCLEIC ACID QUANTITATION USING UV SPECTROPHOTOMETRY

1. DNA and RNA absorb at 260 nm

2. Proteins absorb at 280 nm

3. [DNA]= A_{260} X dilution factor X 50 ug/ml
   1OD unit at 260 nm (A_{260})= 50 ug/ml of DNA

4. [RNA]=A_{260} X dilution factor X 40 ug/ml
   1OD unit at 260 nm (A_{260})= 40 ug/ml of RNA

Concentration= ug of DNA or RNA per ml of hydrating solution

Example 1:

DNA preparation diluted 1:200 yields A_{260} reading of 0.200

DNA concentration (ug/ml) = 0.200 absorbance units X 200 X 50 ug/ml per absorbance unit = 2000 ug/ ml

Example 2:

RNA preparation diluted 1:10 yields A_{260} reading of 0.500

RNA Concentration = 0.500 absorbance unit X 10 X 40 ug/ml per absorbance unit = 200 ug/ml

QUALITY FROM UV SPECTROPHOTOMETRY

1. A_{260}/A_{280}= measure of purity

2. 1.8-2.0 = good DNA or RNA

3. Less than 1.8 = too much protein or other contaminants

CALCULATING NUCLEIC ACID YIELD

1. DNA/ RNA concentration multiplied by volume of hydrating solution

Example: If DNA concentration from UV spectrophotometry = 250 ug/ml

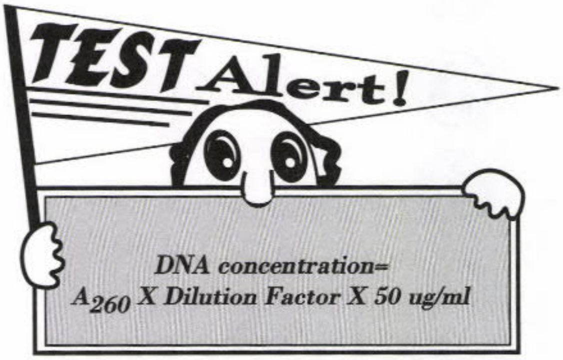
Volume of hydration solution = 0.1 ml

Then DNA yield = 250 ug/ml X 0.1 ml = 25 ug

REMEMBER!

Isolation of DNA from Clinical Specimens

Some = Separate
Lazy = Lyse
Dogs = Denature
Can = Contaminants
Play = Precipitate
Right = Resuspend



DNA concentration=
A_{260} X Dilution Factor X 50 ug/ml

RNA concentration=
A_{260} X Dilution Factor X 40 ug/ml

Nucleic acid purity

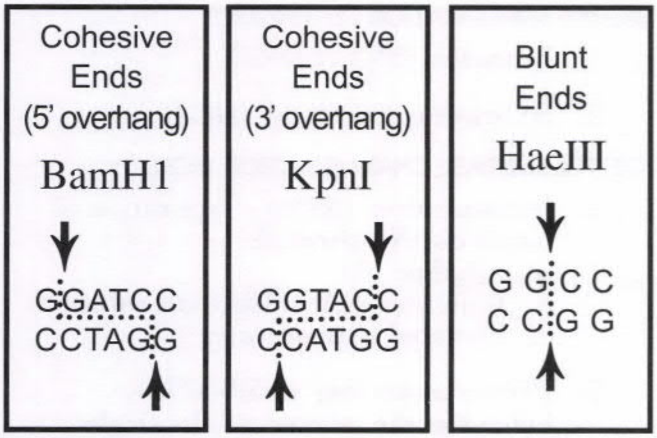
A_{260} / A_{280}

RESTRICTION ENDONUCLEASES

1. Bacterial enzymes cut or nick specific sites of a DNA sequence (*"molecular scalpels"*)

2. Recognize specific short sequences of DNA, usually 4 or 6 bases but some are 5, 8 or longer and cleave at or near recognition site

3. Recognition sequences are palindromes


Cohesive Ends (5' overhang)
BamH1
GGATCC
CCTAGG
Cohesive Ends (3' overhang)
KpnI
GGTACC
CCATGG
Blunt Ends
HaeIII
G G C C
C C G G

REMEMBER!
Palindromes

Palindromes are the same sequence on both DNA strands when read in either direction:

5' – GGTACC – 3'
3' – CCATGG – 5'

DNA SEQUENCING

1. **Determine order of nucleotides in a DNA molecule**
   a. **Maxam- Gilbert (chemical degradation)**
   b. **Sanger method (*dideoxy chain termination*)**
   c. **Pyrosequencing (*sequence by synthesis*)**
   d. **Next generation sequencing (*sequence single molecules of DNA in real time*)**
      - ❖ ***Pacific Biosciences***
      - ❖ ***Oxford Nanopore***
      - ❖ ***Life Sciences Qdot technology***

DNA MICROARRAYS (*DNA CHIP*)

1. **Use hybridization technology to examine gene expression**
2. **Arrangement of DNA sequences on solid support**
3. **Each microarray contains thousands of genes**
4. **Simultaneously monitor gene expression levels in all these genes**
5. **Used for:**
   a. **Gene expression studies**
   b. **Disease diagnosis**
   c. **Pharmacogenetics (*drug discovery*)**
6. **Special instrumentation for**
   a. **Generation of micro arrays**
   b. **Analysis of results**

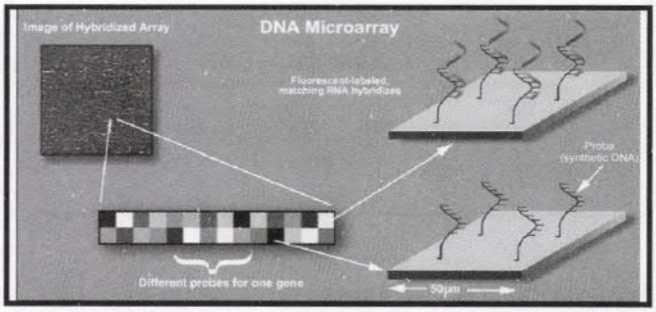
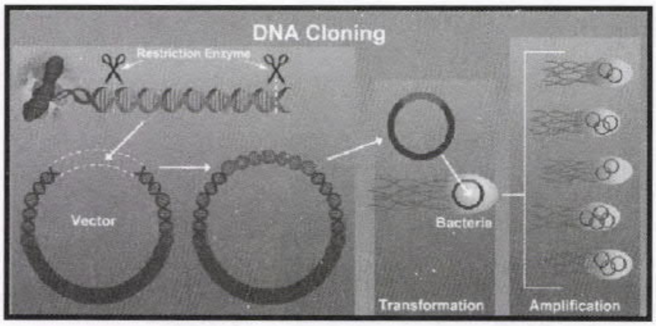


Image of Hybridized Array
DNA Microarray
Fluorescent-labeled, matching RNA hybridizes
Probe (synthetic DNA)
Different probes for one gene
50µm

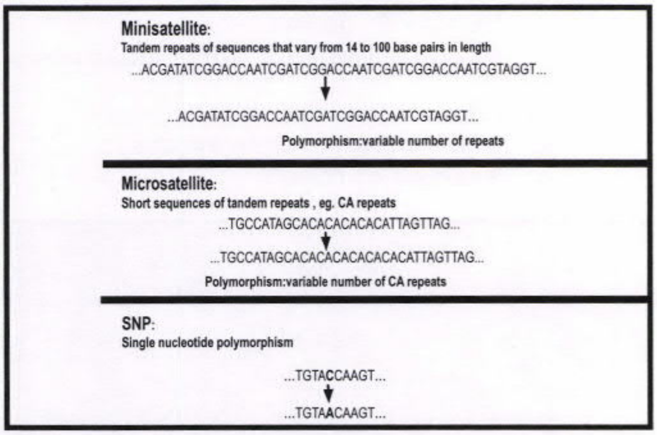
GENE CLONING (*RECOMBINANT DNA TECHNOLOGY*)

1. **Isolating and amplifying a defined DNA sequence**
2. **Uses a vector, such as a plasmid and an appropriate host, such as *E. coli***


DNA Cloning
Restriction Enzyme
Vector
Bacteria
Transformation
Amplification

SINGLE NUCLEOTIDE POLYMORPHISMS (SNP)

1. Mutation of a single nucleotide (*A,C,T,G*)
2. Some associated with various phenotypic differences
    a. Propensity towards disease
    b. Drug resistance
3. Over 5 million SNP locations identified in human genome


Minisatellite:
Tandem repeats of sequences that vary from 14 to 100 base pairs in length
...ACGATATCGGACCAATCGATCGGACCAATCGATCGGACCAATCGTAGGT...
...ACGATATCGGACCAATCGATCGGACCAATCGTAGGT...
Polymorphism:variable number of repeats
Microsatellite:
Short sequences of tandem repeats , eg. CA repeats
...TGCCATAGCACACACACACATTAGTTAG...
...TGCCATAGCACACACACACACACATTAGTTAG...
Polymorphism:variable number of CA repeats
SNP:
Single nucleotide polymorphism
...TGTACCAAGT...
...TGTAACAAGT...

FLUORESCENT IN-SITU HYBRIDIZATION (*FISH*)

1. Hybridization of a fluorescent DNA probe to its complementary DNA in morphologically preserved tissue or cells
2. Detect and localize presence or absence of specific DNA sequence or chromosome
3. Able to examine metaphase chromosome spreads as well as interphase (*non-dividing*) cells

Amplification Techniques

COPY NUMBER AMPLIFICATION

1. Polymerase chain reaction (*PCR*)
2. Ligase chain reaction (*LCR*)
3. Nucleic acid sequence-based amplification (*NASBA*)
4. Transcription-mediated amplification (*TMA*)
5. Strand-displacement amplification (*SDA*)

SIGNAL AMPLIFICATION TECHNIQUES

1. Branched DNA (*bDNA*)
2. Hybrid capture assay (*HCA*)

THE POLYMERASE CHAIN REACTION (*PCR*)

1. Denaturation (*95°C*) – separation of target dsDNA through
    a. Alkaline pH
    b. Ionic strength of high salt solution
    c. Elevated temperature
2. Primer annealing at 50-60°C – hybridize the primers to the single-stranded template
3. Extension (*72°C*) – polymerize the primer into the full-length gene of interest
4. Each cycle doubles amount of DNA

REMEMBER!

Denature by the SEA

Salt (high ionic strength)

Elevated temp

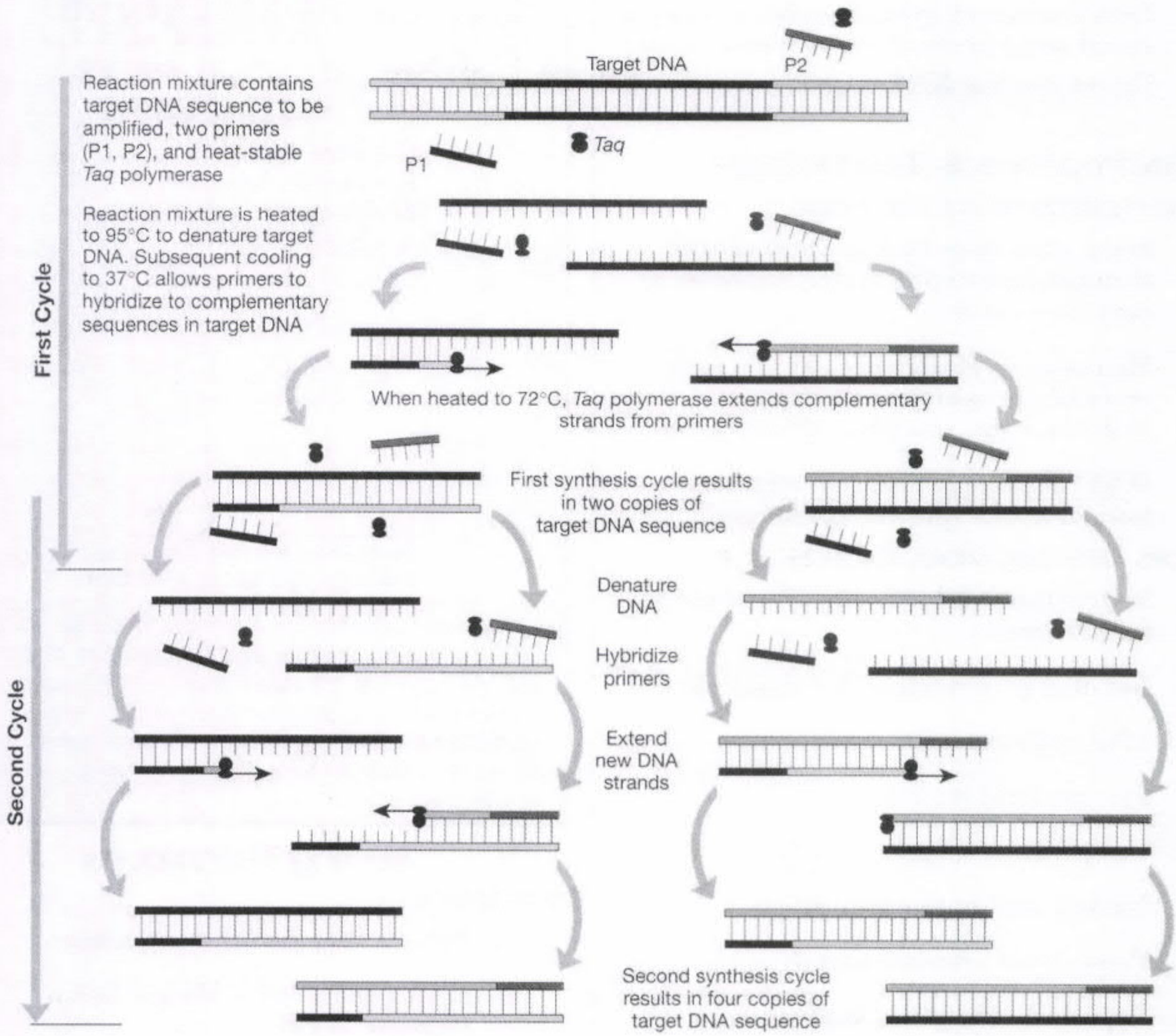
Alkaline pH

CONTROLS FOR PCR

1. Blank reaction
    a. Controls for contamination
    b. Contains all reagents except DNA template
2. Negative control reaction
    a. Controls for specificity of the amplification reaction
    b. Contains all reagents and a DNA template lacking the target sequence
3. Positive control reaction
    a. Controls for sensitivity
    b. Contains all reagents and a known target-containing DNA template

REVERSE TRANSCRIPTION POLYMERASE CHAIN REACTION (*RT-PCR*)

1. Synthesis of complementary piece of DNA (*cDNA*) from RNA by reverse transcription (*RT*)
2. Amplify the cDNA target sequence by PCR


Target DNA
P2
P1
Taq
First Cycle
Second Cycle
Reaction mixture contains target DNA sequence to be amplified, two primers (P1, P2), and heat-stable *Taq* polymerase
Reaction mixture is heated to 95°C to denature target DNA. Subsequent cooling to 37°C allows primers to hybridize to complementary sequences in target DNA
When heated to 72°C, *Taq* polymerase extends complementary strands from primers
First synthesis cycle results in two copies of target DNA sequence
Denature DNA
Hybridize primers
Extend new DNA strands
Second synthesis cycle results in four copies of target DNA sequence

DNA Amplification Using Polymerase Chain Reaction

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REAL-TIME PCR / QUALTITATIVE PCR (*qPCR*)

1. Qualitative and/or quantitative technique
2. Detects fluorescent reporter molecule after each cycle

Strand Displacement Amplification (*SDA*)

1. Target amplification uses heat denaturation, annealing and extension
2. Creates an altered target with a restriction endonuclease recognition site.

NUCLEIC ACID SEQUENCE BASED AMPLIFICATION (*NASBA*)

1. A nucleic acid sequence based amplification technique that amplifies RNA and rRNA
2. Direct detection of RNA viruses / HCV and HIV

HYBRID CAPTURE

1. Nucleic acid amplification technique used for RNA or DNA applications
2. Solid phase using chemiluminescence

BRANCHED CHAIN DNA (bDNA)

1. Uses a series of hybrid probes to elicit a signal amplification- chemiluminescence
2. Detect specific RNA sequences

Electrophoresis Technology

ELECTROPHORESIS OF NUCLEIC ACIDS

1. Separation based on size and charge through a sieve-like matrix (*agarose or polyacrylamide*)
2. Migration in electrical field at a rate inversely proportional to $\log_{10}$ of molecular size *(number of base pairs)*
3. DNA (*negatively charged*) migrates toward anode (*positively charged*)

FACTORS AFFECTING MIGRATION RATE

1. Matrix type and porosity (%) of the gel (*gel casting*)
2. Net charge of nucleic acid molecule
3. DNA conformation
4. Electric field strength
5. Temperature of gel
6. Nucleic acid base composition
7. Presence of intercalating dyes
8. Type and strength of buffer

PULSED FIELD GEL ELECTROPHORESIS OF DNA (*PFGE*)

1. Analysis of DNA fragments up to 100 kb in size
2. Separation accomplished using a pulsed electrical field
3. PFGE commonly used for genotyping prokaryotes

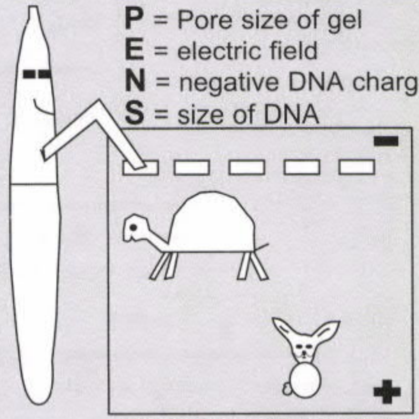
Methods, instruments, reagents & controls

Routine and special procedures to verify test results

REMEMBER!

Tech PENS Load Gels!

P = Pore size of gel
E = electric field
N = negative DNA charge
S = size of DNA



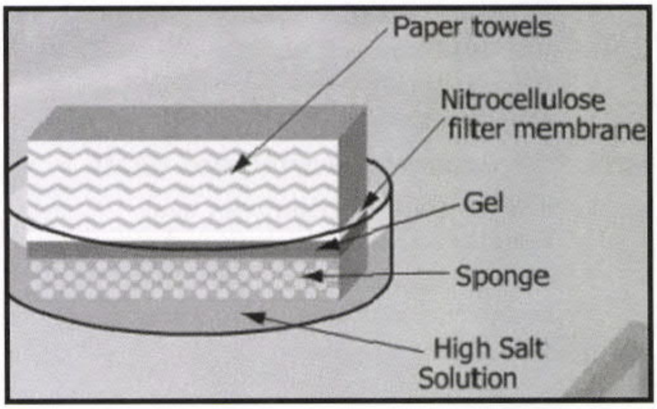
Tortoises are big and slow
Bunnies are small and fast

Movement through a gel depends on the size of the DNA particle, its charge, and the pore size of the gel. All negatively charged DNA particles move toward the anode, but the larger pieces have a harder time squeezing through the small pores in the gel and cannot move as fast, i.e., as far, as the smaller pieces.

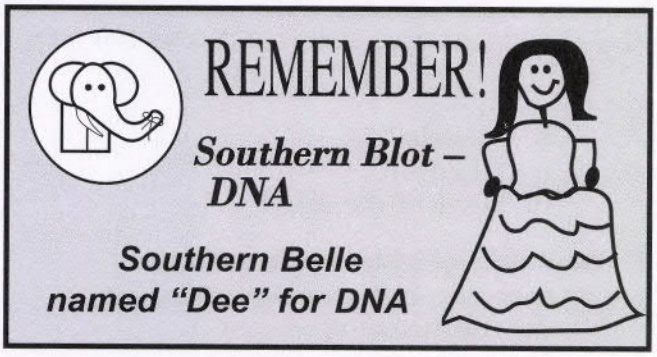
Blotting Techniques

SOUTHERN BLOT

1. Detects specific DNA sequences
2. DNA denatured in the gel by an increase in pH
3. DNA transferred to a membrane by capillary action with a high salt solution
4. Labeled complementary probe used for detection

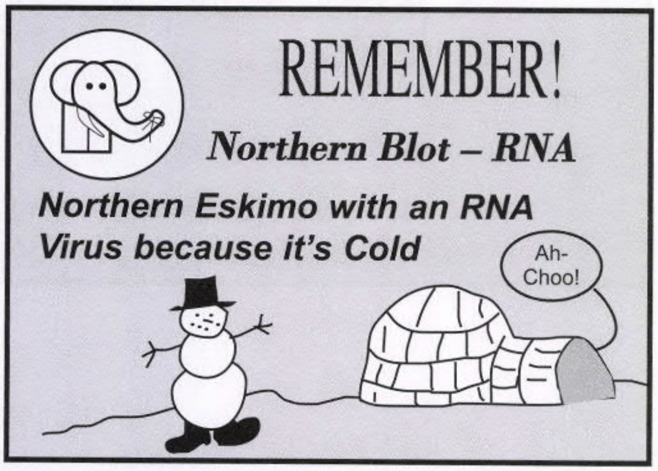


5. Procedure:
   a. Genomic DNA cut with restriction enzymes
   b. DNA electrophoresed
   c. Gel submerged in an alkaline solution to denature the DNA
   d. DNA transferred onto a nitrocellulose membrane by capillary action
   e. Membrane mixed with a solution containing labeled probe
      - ❖ *Probe will hybridize to complementary piece of DNA on gel*
   f. Membrane washed to remove excess, unbound probe
   g. Membrane developed and visualized using either radioactive isotopes, chemiluminescent dyes, or colorimetric techniques


REMEMBER!
Southern Blot – DNA
Southern Belle named "Dee" for DNA

NORTHERN BLOT

1. Detect specific sequences of RNA
2. RNA transferred to membrane by capillary action using a high salt solution
3. Labeled complementary probe used for detection

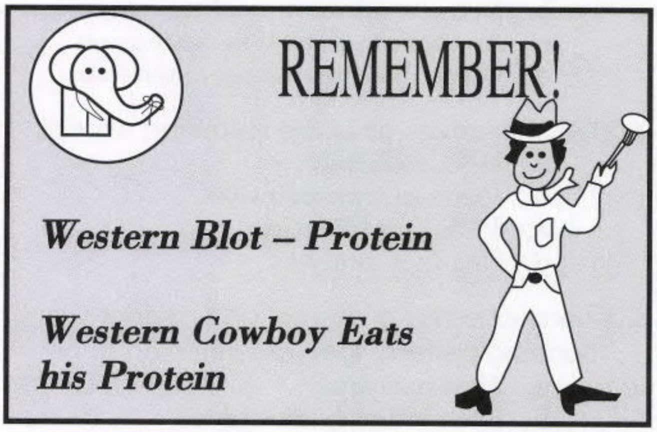

REMEMBER!
Northern Blot – RNA
Northern Eskimo with an RNA Virus because it's Cold
Ah-Choo!

WESTERN BLOT

1. Protein run on SDS- polyacrylamide gel electrophoresis (*PAGE*)
2. Protein electrically transferred to membrane (*electro-transfer*)
3. Membrane incubated with a primary antibody and blocking solution
4. Membrane washed and incubated with secondary antibody and blocking solution
5. Membrane washed and rinsed with substrate buffer
6. Substrate added and developed

SOUTHWESTERN BLOT

1. DNA-binding proteins


REMEMBER!
Western Blot – Protein
Western Cowboy Eats his Protein

DOT/ SLOT BLOTS

1. Quick analysis of DNA and RNA
2. Does not determine the size of target
3. Applied to:
   a. Expression analysis
   b. Mutation anaysis
   c. Amplification analysis

REVERSE DOT BLOTS

1. Reverse allele specific oligonucleotide
2. Hybridization
3. Important method for genotyping common human mutations

STRINGENCY

1. Describes the conditions under which hybridization takes place
2. Salt, heat and formamide increase stringency

MOLECULAR DIAGNOSTICS SAMPLE QUESTIONS

1. Human genome consists of:
    - a. Haploid copy number
    - b. 22 chromosomes
    - c. Circular structure
    - d. Approximately 6 billion bases

2. The migration rate of a macromolecule through a gel matrix during electrophoresis depends on:
    - a. Net charge on the molecule
    - b. Size of the molecule
    - c. Thickness of gel
    - d. All of the above

3. One cycle of a polymerase chain reaction (*PCR*) includes:
    - a. Denaturation, digestion, detection
    - b. Denaturation, annealing, extension
    - c. Annealing, detection, extension
    - d. Digestion, annealing, extension

4. RT-PCR involves all of the following except:
    - a. DNA isolation
    - b. Reverse transcription
    - c. PCR amplification
    - d. Product analysis

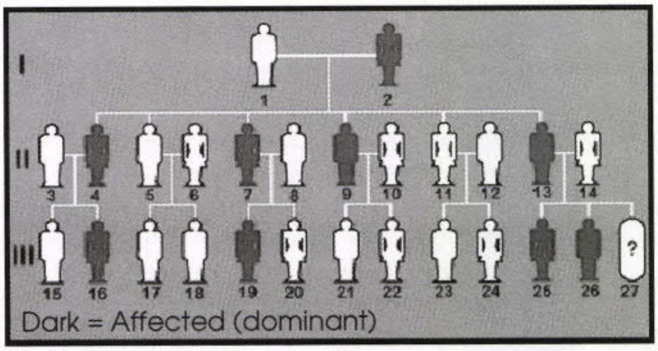
5. If someone has a normal and mutant banding pattern, they are referred to as:
    - a. Heterozygous
    - b. Compound homozygous
    - c. Homozygous
    - d. Wild type

6. The coding sequences of a gene are known as:
    - a. Introns
    - b. Exons
    - c. Splice sites
    - d. Frameshifts

7. Restriction endonucleases recognize specific:
    - a. Methylation patterns
    - b. Trinucleotide repeats
    - c. Palindromic DNA sequences
    - d. DNA-damaged sites

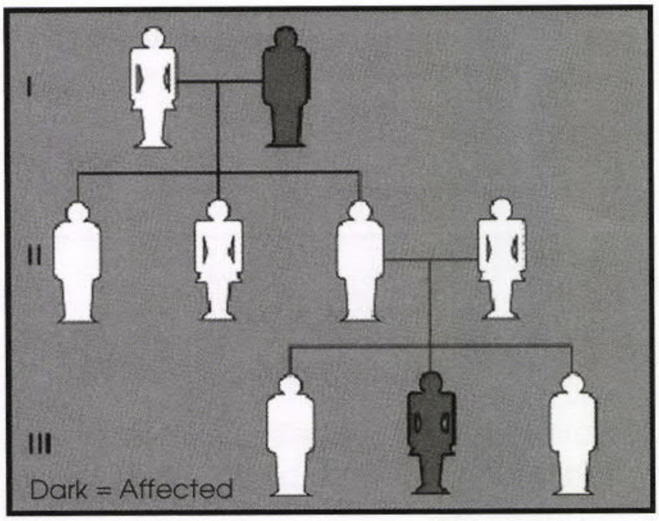
8. Western Blotting is a method used to detect which of the following:
    - a. DNA
    - b. Protein
    - c. RNA
    - d. Mutations

9. What are the chances that individual number 27 will be affected with an autosomal dominant trait?
    - a. 0%
    - b. 25%
    - c. 50%
    - d. 100%



10. X-linked recessive traits are more common in:
    - a. Males
    - b. Females
    - c. Children
    - d. None of the above

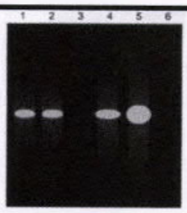
11. What type of inheritance pattern is represented in this pedigree?
    - a. Autosomal dominant
    - b. Autosomal recessive
    - c. X-linked recessive
    - d. X-linked dominant



12. Using the picture below, which is the SNP?
    a. t/a
    b. c/c
    c. c/a
    d. t/g

ATTCGCATGCCTAGTCAAATGC	Abnormal Allele
ATTCGAATGCCTAGTCAAATGC	Normal Allele

13. According to the following western blot, which vegetables contain chlorophyll?
    a. Green bean, carrot and asparagus
    b. Green bean, squash, and asparagus
    c. Green bean, asparagus, and spinach
    d. Green been, carrot and squash



Lane 1 = Control protein - chlorophyll
Lane 2 = Green bean extract
Lane 3 = Carrot extract
Lane 4 = Asparagus extract
Lane 5 = Spinach extract
Lane 6 = Yellow squash extract

14. The absorbance reading at 260 nm for a 1:100 dilution of a DNA sample is 0.430. Which of the following is the correct calculation for the DNA concentration?
    a. 0.172 ug/mL
    b. 0.215 ug/mL
    c. 1720 ug/mL
    d. 2150 ug/mL

15. The absorbance reading at 260nm for a 1:200 dilution of RNA sample is 0.210. Which of the following is the correct calculation for the RNA concentration.
    a. 1680 ug/ml
    b. 16.80 ug/ml
    c. 1.680 ug/ml
    d. 0.168 ug/ml

16. A DNA preparation has the following absorbance readings

A_{260}=0.260

A_{280}=0.230

Based on the ratio, which of the following choices is correct?

The sample is
    a. suitable
    b. contaminated with chloroform
    c. contaminated with phenol
    d. contaminated with protein

17. A DNA sample is isolated from peripheral blood cells of a patient. When performing spectrophotometic analysis to determine the yield of DNA in the sample, you find the 1:50 dilution of the 0.4 ml sample gives an OD_{260} reading of 0.041. What is the total amount of DNA contained in the 0.4 ml sample?

    a. .41 ug
    b. 4.1 ug
    c. 41 ug
    d. 410 ug

ANSWERS AND RATIONALE

1. D

The human genome is dipoid, not haploid. It has a total of 23 chromosome pairs, not 22. It is linear, not circular. But it does have about 6 billion bases.

2. D

The migration rate of a macromolecule through a gel matrix during electrophoresis depends on all three, the thickness of the agarose gel, the net charge of the molecule, and the size of the molecule.

3. B

One cycle of a polymerase chain reaction (*PCR*) includes a denaturation step to break the strand apart, an annealing step so that the primers can sit down on the appropriate area of the DNA, and an extension so that the Taq polymerase can make a copy of the strand of DNA.

4. A

RT-PCR involves PCR amplification of the product, reverse transcriptase is the enzyme that is used in RT-PCR, and the product must be analyzed. However, RT-PCR requires isolation of RNA, not DNA.

5. A

If someone has a normal and mutant banding pattern, they are referred to as heterozygous. Homozygous individuals have identical patterns. Wildtype refers to the "normal" or prototype cell.

6. B

The coding sequences of a gene are known as exons. The introns are spliced out before the gene is translated. The splice site is just the point at which an enzyme can cut the DNA. A frameshift mutation is when a nucleotide is either deleted or added to a sequence such that the protein produced is altered.

7. C

Restriction endonucleases recognize specific 4 – 5 nucleotide palindromic *(reads the same in either direction)* DNA sequences.

8. B

Western Blotting is used to identify proteins through the use of SDS-PAGE. Southern Blotting identifies DNA and Northern Blotting identifies RNA. Mutations are usually identified in the genomic DNA, therefore, a Western would not be used.

9. C

The parent, #13 on the chart, is heterozygous for the dominant trait since his father, #1 on the chart, is homozygous for a recessive trait. Therefore, #27 has a 50% chance of being affected.. If you quickly sketch a Punnett Square Aa x aa for this pedigree – you will see that half will have the trait (*Aa*).

10. A

X-linked traits are always more common in males because males have one X chromosome (*XY*) as opposed to females who have two (*XX*). In females the other X chromosome carries the dominant genes so as carriers, they do not display the trait. Males with only one X chromosome display whatever traits are on that chromosome, dominant or recessive.

11. B

This pedigree is an example of an autosomal recessive trait. In an autosomal recessive disorder, an individual must have two copies of the abnormal gene in order to be affected. Also, autosomal recessive traits commonly skip generations. It is not an X-linked recessive trait because there is male to male transmission – father in generation I would have to have passed it to his son in generation II for him to pass to his daughter in generation III. Fathers can only pass the X linked recessive genes to their daughters *(they get the X chromosome)* not to their sons *(they get the Y chromosome)*.

12. C

A polymorphism is a change in the DNA sequence. In this example, the SNP or single nucleotide polymorhphism is an A in the normal allele that changed to a C in the abnormal allele. If you match the top and bottom sequences, they are identical except for the sixth nucleotide in from the left.

13. C

A Western blot is used to analyze the presence of protein in a sample. Therefore, if the specific protein is present, you will see a product on the Western blot. Lanes 1, 2, 4, and 5 have a product. Lane one is the positive control and should have a product. Lane 2, 4, and 5 are extracts from green bean, asparagus and spinach. Hence, these are the vegetables that contain chlorophyll.

14. D

The calculation to determine the DNA concentration is OD_{260} (*reading of the DNA sample*) multiplied by the dilution factor. multiplied by 50 (*1 OD unit at 260 nm for DNA*)

0.430 x 100 x 50 = 2150 ug/mL

15. A

The calculation to determine the RNA concentration is OD_{260} (*absorbance reading of the RNA sample*) multiplied by the dilution factor, multiplied by 40 (*1 OD unit at 260 nm for RNA*) . Therefore,

0.210 X 200 X 40 ug/ml = 1680 ug/ml

16. D

To determine whether a DNA sample is contaminated or not, you divide the A260 by A280. For DNA, if the ratio is <1.8 the sample is contaminated with protein. A ratio of 1.8-2.0 indicates high purity.

0.260 /0.230 = 1.1

1.1 is less than 1.8

DNA sample is contaminated with protein

17. C

First calculate the DNA concentration then multiply it by the hydrating volume.

DNA concentration=

0.041 X 50 ug/ml X 50 =102.5 ug/ml

DNA yield = 102.5 ug/ml X 0.4 ml

= 41 ug

References

Tsongalis, Gregory T. and William B. Coleman. *Molecular Diagnostics A training and study guide.* Copyright 2002. American Association for Clinical Chemistry Press. Washington, DC.

Roche Diagnostics. *Molecular Technology Education Program.* Copyright 2010 CD-rom version 6.0. Go to www.rochegenetics.com to order a copy of the CD.

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LABORATORY OPERATIONS AND INSTRUMENTATION

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Quality Assurance

1. QA takes into consideration all factors that may affect the treatment of a patient
   a. Pre-analytical variables
      - ❖ *Specimen collection issues*
      - ❖ *Transport*
      - ❖ *Preservatives used*

   b. Analytical variables
      - ❖ *Test processing*
      - ❖ *QC data useful here*

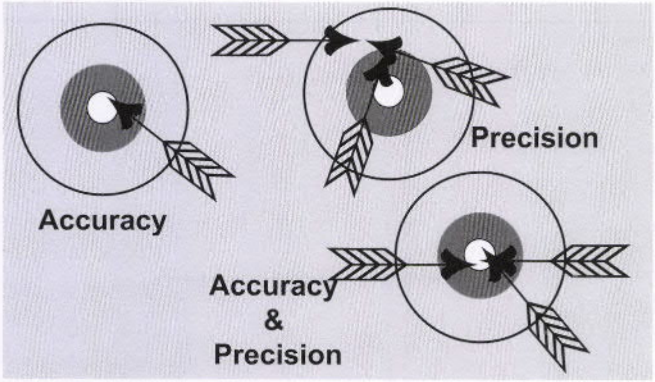
   c. Post-analytical variables
      - ❖ *How results are reported and acted upon*

Quality Control

DEFINITIONS

1. Accuracy - agreement of results with true value for substance in given specimen
   a. Statistical measures of accuracy
      - ❖ *Mean ($\bar{x}$)= average*
      - ❖ *Mode = most common value*
      - ❖ *Median = middle value*

2. Precision - ability to produce series of results on the same sample that agree closely with each other (*reproducibility*)



Determine Accuracy and Precision from Data

   a. Statistical measures of precision
      - ❖ *Standard deviation (s or SD)*

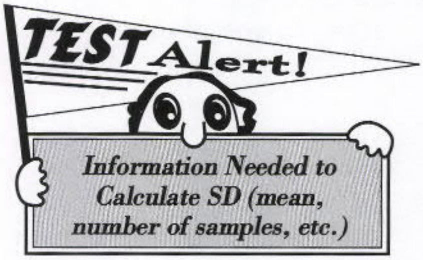
$$S = \sqrt{\frac{\Sigma (X - \bar{x})^2}{n - 1}}$$

Σ = sum
n = number of values
X = individual value
$\bar{X}$ = mean

      - ❖ *Coefficient of variation (CV) (Relative Standard Deviation) Expressed as percent*

$$CV (\%) = \frac{S * (100)}{\bar{X}}$$

   b. Minimum of 20 analyses are needed to determine standard deviation.
   c. Normal distribution *(Gaussian)*
      - ❖ *68% of values will fall within ± 1s*
      - ❖ *95% of values will fall within ± 2s*
      - ❖ *99% of values will fall within ± 3s*



Information Needed to Calculate SD (mean, number of samples, etc.)

Correlate % CV and Standard Deviation

3. Sensitivity - ability to detect small concentrations of a substance

4. Specificity - ability to measure only the substance of interest (*no interfering substances)*

5. Reference range (*normal range*)- range in which 95% of the population is expected to fall; random samples from a given "normal" population will fall within the mean ± 2s range of values for that population

6. **Standards - substances whose exact concentrations, purity or quality are known**
   a. **Primary standard - chemically pure, can be weighed or measured directly**
   b. **Secondary standard - assayed value established by a reference method or comparison to a solution of known concentration *(less expensive)***

7. **Controls - substances having a known or determined "range" of values**
   a. **Assayed - values stated by manufacturer**
   b. **Unassayed values not given, determined by user**

8. **Confidence interval *(control range)* - range of values within which control result must fall**
   a. **Usually established by analyzing the control at least 20 times**
   b. **Calculate mean, standard deviation, and ±2s range**

9. **Delta check - compare most recent patient result with previous result(s)**
   a. **Large disparity usually indicates error in one of the results**

10. **Comparison to external quality control**
    a. **Standard Deviation Index (SDI)**

$$\text{SDI} = \frac{\text{Your Lab's Result} - \text{Group } \bar{x}}{\text{Group Standard Deviation}}$$

SDI should be less than 2

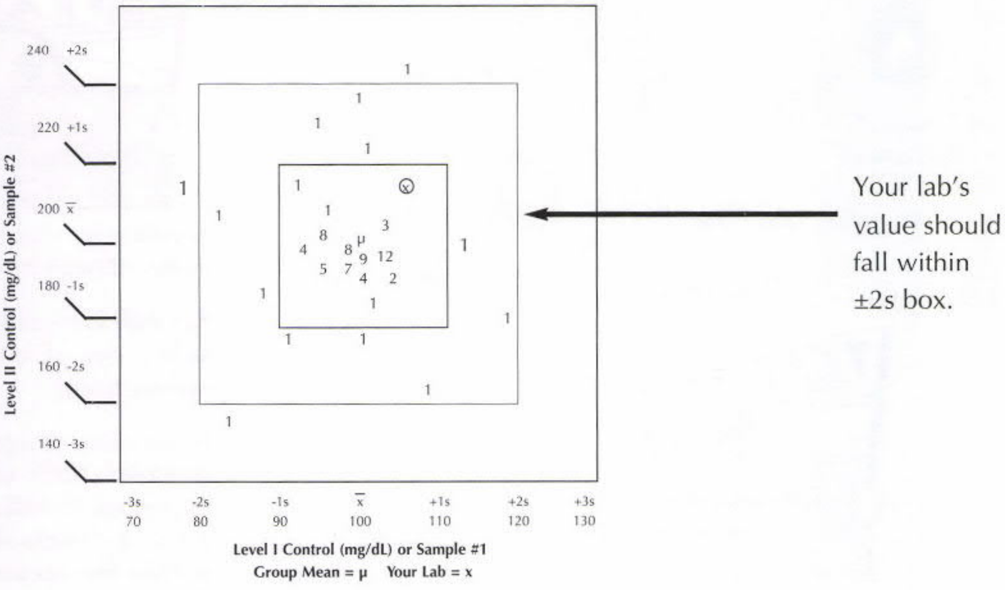
    b. **Youden plot *(see below)***
       - ❖ ***Plot Level I control vs Level II control***

11. **Sources of Variation or Error *(see diagram next page)***
    a. **Random error - may be due to: instrument errors *(voltage fluctuations, incorrect sample volume)* sample error (*anticoagulant or drug interference, lipemia, hemolysis*), or human error (*preparation or storage of control samples)***
    b. **Systematic error - may be due to: instrument errors (*dirty photometer, faulty ISE)*, decomposition of standards or reagents (*evaporation or contamination)***

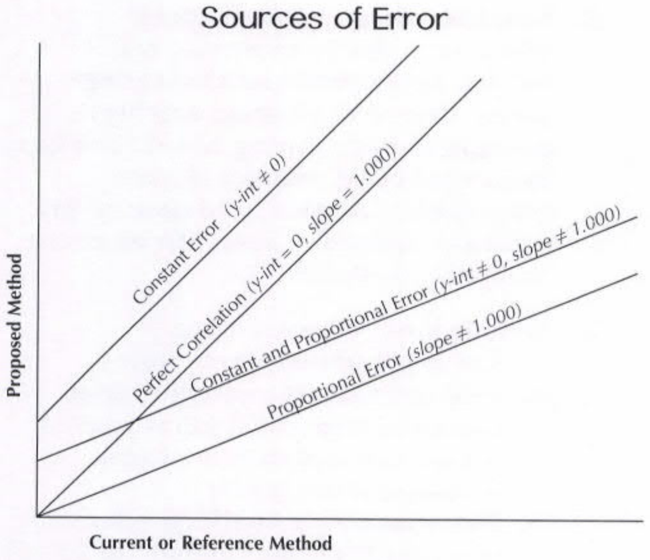
Systematic errors may be constant bias (*y-intercept is not zero)* or proportional bias *(slope is not 1.000)*

Verify Test Results with Delta Check

EXAMPLE OF YOUDEN PLOT



Your lab's value should fall within ±2s box.



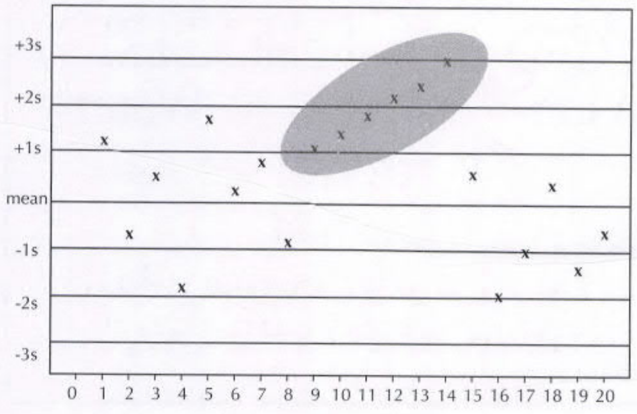
12. t-test - comparison of the means of two populations or two test method means, used to compare the *accuracy* of two methods.

13. F-test - comparison of standard deviations of two populations or two test method standard deviations, used to compare *precision* of two methods

TEST Alert!
Purpose of t-Test

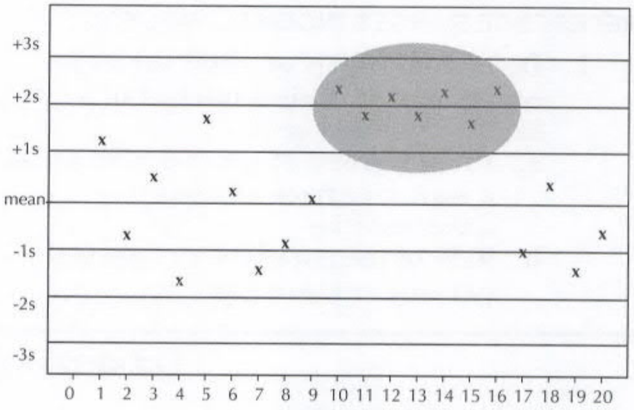
INTERPRETATION OF QC

1. Trend - values for the control that continue to either increase or decrease over a period of 6 consecutive days (*a trend may be due to reagent deterioration or instrument wear*) - Determine by visual inspection of Levey-Jennings quality control chart



Trend (Days 9-14)

2. Shift - 6 or more consecutive daily values that distribute themselves on one side of the mean value line, but maintain a constant level *(a shift may be due to deterioration of a standard or a change in reagent lot)* - Determine by visual inspection of Levey-Jennings quality control chart



Shift (Days 10-16)

3. Westgard Multi-Rules - used to accept or reject a "run" of samples
   a. Warning Rule -
      1:2s - Evaluate previous controls when one observation exceeds the mean ±2s limit. If no other rules are broken, accept results

   b. Reject Rules (*Out-of-Control*)
      1:3s - Reject when one observation exceeds the mean ±3s limit

      2:2s - Reject when two consecutive observations exceed the same mean +2s limit or the same mean -2s limit

      R:4s - Reject when one control observation in the run exceeds its mean +2s limit and the next exceeds its mean -2s limit *(The two points are more than 4s apart)*

      4:1s - Reject when four consecutive control observations exceed the same mean +1s limit or the same mean -1s limit

      10x - Reject when ten consecutive control observations fall on one side of the mean

TEST Alert!
Evaluate Data to Determine Shifts and Trends

REFERENCE RANGES (NORMAL VALUES)

1. Definition - range of usual values for constituent of clinical interest in a healthy population
   a. From a group of persons who are in a state free from obvious abnormalities
   b. 95% of the population of normal persons or mean $\pm 2s$
2. Considerations - physiological differences due to race, age, sex, weight, nutritional and absorptive states, degree of physical activity, position of body during blood sampling, menstrual cycle, emotional state, geographic location, and time of day (*diurnal variation)*; also differences in analytical methods
3. Establishing reference ranges
   a. Large population preferable
   b. (100 individuals minimum) from community in which lab is located
   c. Values for analyte must follow Gaussian distribution
   d. Determine 95% confidence limits $(\bar{x} \pm 2s)$ (*1 "normal" person in 20 will fall outside of this range*)

Laboratory Mathematics

MEASURING CONCENTRATION

1. Can be measured in one of three ways:
   a. Weight per unit weight (w/w)
   b. Weight per unit volume (w/v)
   c. Volume per unit volume (v/v)
2. A common way to express concentration is in per cent (%), which simply means parts per 100

WEIGHT PER UNIT WEIGHT (W/W)

1. Most accurate type of % concentration; not often used in the clinical laboratory
2. Example:
   Make a 30% w/w NaCl aqueous solution

   Answer:
   Mix 30 gm of NaCl with 70 gm H_20
3. Example:
   Make 100 gm of a 30% NaCl aqueous solution

   Answer:
   30% of the total must be NaCl
   100 gm X .30 = 30 gm
   To make 100 gm of this solution, mix 30 gm NaCl with 70 gm H_20

WEIGHT PER UNIT VOLUME (W/V)

1. Most commonly used method in the clinical laboratory; a solid solute is mixed with a liquid solvent
   (1 mL H_20 = 1 gm H_20) (1% = 1 gm/100mL)
2. Example:
   Prepare 100 ml of a 0.5% NaCl solution

   Answer:
   Measure 0.5 gm NaCl and add H_20 up to 100 mL

VOLUME PER UNIT VOLUME (V/V)

1. When a solution has a liquid solute in a liquid solvent
2. Example:
   Prepare a 25% H_2SO_4 solution

   Answer:
   A 25% H_2SO_4 solution = 25 mL of concentrated H_2SO_4 in 100 mL of solution. Add 25 mL of concentrated acid to 75 mL H_20

REMEMBER!
Acid to Water
NEVER add water
to acid.
Do what you "oughta", ADD
THE ACID TO THE "WATA".

CHANGING CONCENTRATION AND ACID-BASE NEUTRALIZATION

1. **The volume of one solution times the concentration of that solution equals the volume of a second solution times the concentration of the second solution**

$$V_1 \text{ X } C_1 = V_2 \text{ X } C_2$$

(Units on both sides of the equation must be equal.)

2. **Example:**

How much 95% alcohol is required to make 200 mL of 5% alcohol?

Answer:

$$V_1 \text{ X } C_1 = V_2 \text{ X } C_2$$
$$200 \text{ X } 5 = V_2 \text{ X } 95$$
$$1000 = 95\ (V_2)$$
$$V_2 = 10.5$$

(10.5 mL of 95% alcohol diluted up to 200 mL)

NOT CHANGING CONCENTRATION

1. **Involves variation in the amount of total solution.**

2. **Easiest method is the ratio-proportion procedure.**

$$\frac{\text{unit weight 1}}{\text{unit volume 1}} = \frac{\text{unit weight 2}}{\text{unit volume 2}}$$

Cross-multiply to solve.

3. **Example:**

Prepare 500 mL of 0.5% NaOH.

Answer:

$$\frac{0.5 \text{ gm NaOH}}{100} = \frac{X}{500}$$

$$100X = 500\ (.5)$$
$$100X = 250$$

X = 2.5 gm NaOH diluted up to 500 mL

Example:

A solution contains 45 gm of solute in 240 mL of solution. What is the % concentration?

Answer:

$$\frac{45 \text{ gm}}{240} = \frac{X \text{ gm}}{100}$$

$$240X = 4500$$

X = 18.75 gm per 100 mL = 18.75% solution

MOLARITY

1. **A mole of a substance = number of grams equal to the atomic or molecular weight of the substance**

2. **Gram molecular weight (GMW) is often used as a definition of mole; a 1 molar solution contains 1 mole of solute per liter of solution**

3. **Example:**

What is the GMW of H_2SO_4?

Answer:

2 H = 1 X 2 = 2
1 S = 32 X 1 = 32
4 O = 16 X 4 = 64

GMW = 98

4. **Molarity (M) expresses the number of moles of substance in 1 liter (1000 mL) of solution.**
   a. **Molarity (M) = # of moles/liter**
   b. $$M = \frac{\text{grams/liter}}{\text{GMW}}$$
   c. # grams/liter = GMW X M
   d. $$M = \frac{\% \text{ X } 10}{\text{GMW}}$$
   e. % X 10 = GMW X M
   f. Other units (mmoles and mM)
      1 mmole=1/1000 mole

$$\text{mM} = \frac{\text{mg/liter}}{\text{GMW}}$$

5. **Example:**

Make 500 mL of 2M NaCl.

Answer:

Atomic wt of Na = 23
Atomic wt of Cl = 35.5
GMW = 58.5

GMW X M = # gms/L
58.5 X 2 = 117 gm/L

Dilute 117 gms NaCl up to 1000 mL. Use ratio-proportion to adjust total volume to 500 mL.

$$\frac{117 \text{ gm}}{1000 \text{ mL}} = \frac{X \text{ gm}}{500 \text{ mL}}$$

1000X = 58500

X = 58.5 gms NaCl diluted to 500 mL

6. **Example:**
   What is the molarity of a solution in which there are 25 gms of Na_2SO_4 in 500 mL solution?

   Answer:

   2 Na = 2 X 23 = 46
   1 S = 1 X 32 = 32
   4 O = 4 X 16 = 64
   GMW = 142

   $$M = \frac{gm/L}{GMW}$$

   First, use a ratio-proportion to change 500 mL to 1 L.

   $$\frac{25\ gms}{500\ mL} = \frac{X\ gms}{1000\ mL}$$

   X = 50 gms/L

   $$M = \frac{50}{142} = .35\ M$$

NORMALITY

1. **Based on the same principle of molarity except M is based on GMW and N is based on gram equivalent weight (*GEW*)**
2. **As a general rule, the GEW of a substance is equal to the GMW divided by the valence; GEW is always equal or less than GMW**
3. **Gram equivalent weight (*GEW*) = gram molecular weight/valence**
4. $\text{Normality (N)} = \frac{\text{\# grams/liter}}{GEW}$
5. # grams/liter = GEW X N
6. $N = \frac{\% \times 10}{GEW}$
7. % X 10 = GEW X N
8. **Example:**
   Make 200 mL of a 0.5 N H_2SO_4 solution.

   Answer:

   2 H = 2 X 1 = 2
   1 S = 1 X 32 = 32
   4 O = 4 X 16 = 64
   GMW = 98

   $$GEW = \frac{GMW}{valence} = \frac{98}{2} = 49$$

   gms/L = GEW X N

   gms/L = 49 X 0.5 = 24.5 gms/L

   $$\frac{24.5\ gms}{1000\ mL} = \frac{X\ gms}{200\ mL}$$

   X = 4.9 gms of H_2SO_4 diluted up to 200 mL

CONVERSION OF MOLARITY TO NORMALITY

1. N = M X valence
2. $M = \frac{N}{valence}$
3. **Example:**
   Express 0.4 N H_3PO_4 as molarity.
   $M = \frac{0.4}{3} = .13$

CONVERSIONS BETWEEN MEQ/DL AND MG/DL

1. **mg/dL means mg/100 mL; multiply the number of mg/100 mL by 10 to get the number of mg/1000 mL**
2. $mEq/L = \frac{mg/L}{GEW} = \frac{mg/100mL \times 10}{GEW}$

   $mEq/L = \frac{mg/dL \times 10}{GEW}$
3. $mg/dL = \frac{mEq/L \times GEW}{10}$
4. **Example:**
   Express 200 mg/dl Cl as mEq/L

   Answer:
   $mEq/L = \frac{200 \times 10}{35.5} = \frac{2000}{35.5} = 56.34\ mEq/L$

NOTES

1. mg% = mg/dL
2. % = parts/100 = gm/100 mL
3. 1 gram mol. wt. (GMW) = 1 mole
4. 1 mg. mol. wt. = 1 mmole
5. 1 mole = 1000 mmole
6. M = mmole/mL
7. mM/L = mEq/L

8. Molarity is based on grams per 1000 mL of solution; for any volume other than 1000 mL, use a ratio/proportion formula to solve the problem

TEST Alert!
Preparing Standard Solutions

Conversion to Decimals

Prefix	Symbol	Decimal
Primary Unit	none	1.0
Milli	m	0.001
Micro	µ	0.000001
Nano	n	0.000000001
Pico	p	0.000000000001
Femto	f	0.000000000000001

Conversion Between Units

Multiple	Mass	Volume
One (std)		Liter (L)
$^{1}/_{10}$		Deciliter (dL)
$^{1}/_{1000}$	Milligram (mg)	Milliliter (mL)
$^{1}/_{1,000,000}$	Microgram (µg)	Microliter (µL)
$^{1}/_{1,000,000,000}$	Nanogram	
$^{1}/_{1,000,000,000,000}$	Picogram (pg)	

Example:
Convert µL to mL
Step 1: µL = .000001 = 10^{-6}
mL = .001 = 10^{-3}
Step 2: $10^{-6} - 10^{-3} = 10^{-3} = .001$
Step 3: 1 µL x .001 = .001 mL

TEST Alert!
Conversion of mL to µL

Chemistry / Immunology Instrumentation

SPECTROPHOTOMETRY

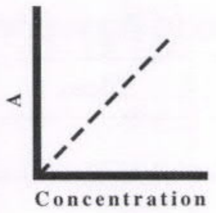
1. Principle - measurement of light in a narrow wavelength range; wavelength selected by prisms, gratings or filters
   a. Chemical reaction produces a substance that absorbs light
   b. Measurements may be made in the visible range (*most common*), ultraviolet range or infrared region
   c. Under suitable conditions, the amount of light absorbed by a solution when illuminated with light of a proper wavelength, is directly proportional to the concentration of its color component (*Beer's Law*)
   d. Basically, the same principle for all analytical methods that measure radiation (*Ex. gamma counters)*

2. Mathematical formula for Beer's law:

$$A = abc = \log \frac{100}{\%T} = 2 - \log \%T$$

A = absorbance
a = absorptivity
b = light pattern of the solution in cm
c = concentration of the substance
%T = transmittance

   a. If measurement is made in absorbance, plot on linear graph paper; concentration of substance is directly proportional to amount of light absorbed



b. **Beer's law is usually used to measure absorbance and calculate the concentration by rearranging the formula (*previous page*):**

$$c = \frac{A}{ab}$$

where a and b are constants

c. **Ratio of standard to unknown - the simplest type of concentration measurement involves determination of the absorbance values for a known concentration of the substance measured (*standard*) and the measurement of that substance in a patient or control sample (*unknown*)**

$$\text{If Abs. of std.} = abc(\text{std.})$$

and

$$\text{Abs. of Unk.} = abc(\text{unk.})$$

then

$$\frac{\text{Abs. of std.}}{\text{Abs. of unk.}} = \frac{abc(\text{std.})}{abc(\text{unk.})}$$

or

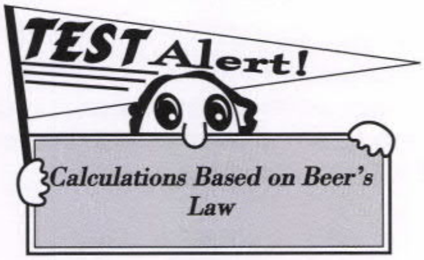
$$\frac{\text{Abs. of std.}}{\text{Abs. of unk.}} = \frac{c(\text{std.})}{c(\text{unk.})}$$

or

$$c(\text{unk.}) = \frac{\text{Abs. of unk.}}{\text{Abs. of std.}} \times c(\text{std.})$$

Example:
Unknown absorbance = 0.252
Standard absorbance = 0.640
Conc. of standard = 200 mg/dL

$$c(\text{unk.}) = \frac{0.252}{0.640} \times 200 = 79 \text{ mg/dL}$$


TEST Alert!
Calculations Based on Beer's Law

d. **Deviations from Beer's Law**
- ❖ *Simultaneous absorption at multiple wavelengths*
- ❖ *Absorption of light by interfering substances*
- ❖ *Light transmission by stray light*
- ❖ *Measurement of very high concentrations (dilute sample and multiply results by dilution factor)*

3. **Components of a Spectrophotometer**
   a. **Light source**
      - ❖ *Visible range - tungsten lamp*
      - ❖ *Ultraviolet range - hydrogen discharge or deuterium lamp*

   b. **Monochromator**
      - ❖ *Filter*
      - ❖ *Diffraction grating - range from near-UV to near-infrared*
      - ❖ *Prism - quartz for visible and UV range; glass for visible light only*

   c. **Cuvette - glass, quartz or plastic**
   d. **Photomultiplier tube detector**
   e. **Measuring device/meter**

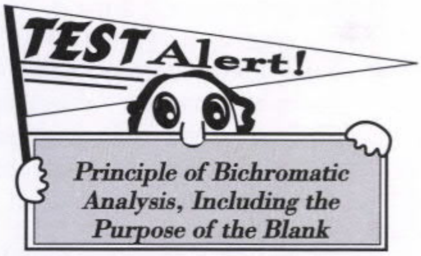
4. **Standardization of Spectrophotometers**
   a. **Wavelength calibration**
      - ❖ *H^+ or deuterium lamps have built-in sources for checking wavelength accuracy*
      - ❖ *Holmium oxide or didymium filters may be used to check wavelength calibration*
      - ❖ *Solutions of stable chromagens can be used to determine if wavelength accuracy of instrument has changed after wavelength accuracy has been determined by one of preceding primary methods*

   b. **Stray *Light***
      - ❖ *Detected by using filters or solutions with sharp cutoff wavelength for transmission (Ex. $NiSO_4$)*
      - ❖ *Corrected by changing light source, verifying wavelength calibration, sealing light leaks, realigning instrument components or cleaning optical surfaces*

TEST Alert!
Spectrophotometer Maintenance & Adjustment

5. **Bichromatic Analysis**
   a. **Automation procedure in which absorbance is measured at two different wavelengths**

  b. Minimizes background interference *(such as hemolysis or icteria)* by providing a blank for each specimen

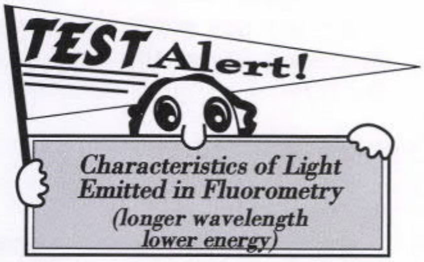


Principle of Bichromatic Analysis, Including the Purpose of the Blank

Sources of Error in Spectrophotometry

FLUOROMETRY

1. Principle
   a. Energy emission that occurs when certain compounds absorb electromagnetic radiation, become excited and return to energy levels slightly lower than their original energy levels
   b. Emitted energy is less than absorbed energy, so the wavelength of emitted light is longer than wavelength of absorbed light
   c. Extremely sensitive, up to 1000 times as sensitive as colorimetric methods
   d. Disadvantage is quenching interference (*occurs when the excited molecule interacts with a substance in the solution and loses some of its energy)*



Characteristics of Light Emitted in Fluorometry (longer wavelength lower energy)

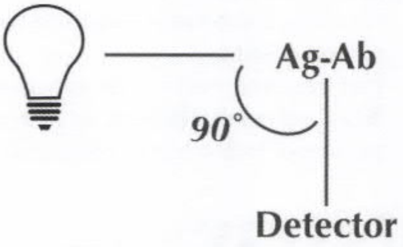
TURBIDIMETRY

1. Principle - measures the amount of light blocked by particulate matter as light passes through the cuvette by a colorimeter or spectrophotometer

2. Sources of Error
   a. Particle size of standard is not the same as the particle size in samples
   b. Particles may settle out as measurements are being made

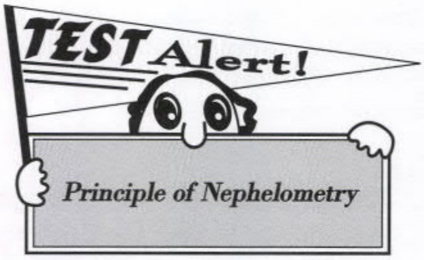
NEPHELOMETRY

1. Principle
   a. Measures light that is scattered by small particles at right angles to the beam incident to the cuvette



   b. Amount of scatter is related to the number and size of the particles
   c. More precise than turbidimetry

2. Used to measure antigen-antibody complexes *(IgG, IgA, IgM, CRP, RF, C3, C4, haptoglobin, etc.)*; provides high specificity



Principle of Nephelometry

OSMOMETRY

1. Osmolality – measure of total number of dissolved particles in a solution (*molecular weight, size, density or type of particle does not matter)*

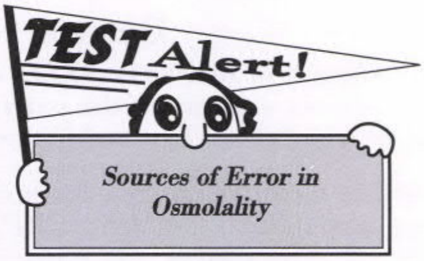
2. Any substance dissolved in a solvent will do the following (*called colligative properties*)
   a. Depress the freezing point
   b. Elevate the boiling point
   c. Decrease the vapor pressure
   d. Increase the osmotic pressure

3. Most practical methods for measuring the concentration of dissolved particles are freezing point depression (*most common method*) and vapor pressure depression

4. Significant differences between methods is a means of determining if a volatile solute such as alcohol is present
   a. Osmolality is increased in the presence of ethanol, methanol and glycol by the freezing point depression method only

   b. Vapor pressure techniques do not measure volatiles (therefore, lower values would be seen with this method)

5. Sources of error
   a. Cooling bath temperature too cool or too warm
   b. Particulate matter in sample does NOT allow sufficient supercooling to occur before freezing takes place


TEST Alert!
Sources of Error in Osmolality

CHROMATOGRAPHY

1. Principle - Separates mixture into individual components on basis of specific differences in physical characteristics

2. Types
   a. Liquid-liquid chromatography - separation is based on differences in solubility between two liquid phases - one aqueous, one organic
   b. Ion exchange chromatography - separation depends on the molecular weight, size and charge of the ions or molecules *(ions with the greater charge densities are held most strongly on ion exchange material)*
   c. Gas-liquid chromatography (*GLC*) and gas chromatography (*GC*) - separation depends on sample volatility and rate of diffusion into liquid layer (partition coefficient) or inert gas (mobile phase)
      - *Retention time is used to identify volatiles (methanol, isopropyl alcohol), drugs, organic acids in urine and catecholamines*
      - *Best method for blood alcohol*
      - *Confirmatory testing - GC with mass spectrometry (MS) required for regulated drugs of abuse*
   d. Thin-layer chromatography (*TLC*) - separation depends on rate of diffusion and solubility of the substance in the solvents as the components migrate through media
      - *Used to identify drugs, lipids, carbohydrates and amino acids*
      - $Rf = \frac{\text{distance moved (constituent)}}{\text{distance moved (solvent)}}$
   e. High-performance liquid chromatography (*HPLC*)
      - *Aqueous or organic solutions are pumped through columns under high pressure, which allows high resolution with fast and accurate quantitation*
      - *Trouble shooting:*
         - Recorder- noisy baseline may be due to bubbles or particulate matter entering the flow cell or a leaking filter; drifting baseline can result from an overloaded column or from contamination in solvent reservoir
         - Loss in column resolution may be due to overloading column with sample or degradation of column packing

ELECTROPHORESIS

1. Principle
   a. Method for the physical separation of proteins based on their ionic charge and molecular size
   b. When placed in an electrical field, electrically charged molecules migrate in a direction that depends on the isoelectric point of the molecule and the pH of the buffer
   c. Factors that affect the rate of migration are molecular weight and size, ionic strength of the buffer and the type of support medium

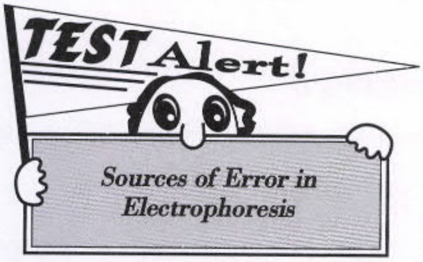
2. Types of electrophoresis
   a. Paper
   b. Agarose gel
   c. Cellulose acetate (*most common)*
   d. Polyacrylamide gel
   e. Starch gel
   f. Isoelectric focusing

3. Components commonly separated
   a. Amino acids
   b. Serum proteins (*see page 108*)
   c. Lipoproteins
   d. Glycoproteins
   e. Nucleic acids
   f. Hemoglobins
   g. Isoenzymes (*CK, ALP*)

h. Immunoglobulins
i. Specific antigens by immunological electrophoretic techniques such as rocket IEP or immunofixation

Sources of Error

PROBLEM	LIKELY CAUSE:
No Migration	Instrument Not Connected Wrong pH Electrodes Connected Backwards
Bands Too Large/Small	Over/Underloading
Very Slow Migration	High Molecular Weight Low Charge Ionic Strength Too High Voltage Too Low
Sample Precipitates in Support	pH Too High or Low Voltage Too High
Split or Broken Bands	Excessive Pressure Applied to Wire Applicators
Crescent-Shaped Bands	Bent Applicators Overloading


TEST Alert!
Sources of Error in Electrophoresis

ELECTROCHEMISTRY (POTENTIOMETRY, AMPEROMETRY, COULOMETRY)

1. Potentiometry - pH meters, ion-selective electrodes (*ISEs*)
    a. Based on the measurement of potential (*voltage*)
    b. Difference between reference and indicator electrodes when no current is passing through the cell; changes in voltage occur in proportion to ionic activity of the solution in which the electrodes are immersed
    c. Reference electrode is usually either saturated calomel (*$HgCl_2$ and KCl*) or silver/silver chloride (*Ag/AgCl*)
    d. Indicator electrode should interact with the analyte of interest and not with other compounds
    e. pH electrodes- glass sensitive to H^+
    f. Na^+ electrodes - glass sensitive to Na^+
    g. K^+ electrodes - liquid ionophore, valinomycin
    h. Gas-sensing electrodes - the membrane is permeable to the gas measured; the CO_2 electrode for the measurement of blood pCO_2 is a pH glass electrode that has a silicone rubber membrane which is permeable to CO_2

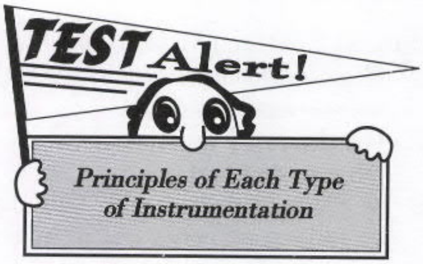
$$CO_2 + H_2O <====> H_2CO_3 <====> H^+ + HCO_3^-$$

H^+ is the measured gas

    i. Enzyme electrodes - electrodes are covered by a layer of immobilized enzymes that catalyze a chemical reaction measured by the electrode

2. Amperometry
    a. Measurement of the current flowing through an electrochemical cell when a constant electric potential is applied to the electrodes
    b. pO_2 electrode for blood gases; cathode is made of platinum, anode is Ag/AgCl
    c. Current directly proportional to the pO_2 in solution is observed

3. Coulometry
    a. Titration in which the titrant is electrochemically generated
        - ❖ *Silver ions are produced by electrolysis from a silver wire used as an anode*
        - ❖ *Chloride ions from the sample complex with silver (Ag^+)ions*
        - ❖ *Indicator electrode senses the excess silver ions*
        - ❖ *When an excess of Ag^+ is detected, the titration stops*
    b. Length of time that it takes for the titrator to generate excess Ag^+ is directly proportional to the Cl^- concentration because the current has been kept constant


TEST Alert!
Principles of Each Type of Instrumentation

Summary of Instrumentation Principles

INSTRUMENTATION	**MEASURES:**
Spectrophotometry	Light in a **Narrow** Wavelength Range
Bichromatic Analysis	Light Absorbence at **2** Different **Wavelengths**
Fluorometry	Light Emitted from Compounds that **Absorb Electromagnetic Radiation**, Become Excited and Return to Energy States Slightly Lower than Their Original Energy States
Turbidimetry	Light **Blocked** by Particulate Matter as Light Passes Through the Cuvette by a Colorimeter or Spectrophotometer
Nephelometry	Light **Scattered** by Small Particles at **Right Angles** to the Beam Incident to the Cuvette
Osmometry	Total **Number of Dissolved Particles** in a Solution Based on Colligative Properties *(freezing point depression and vapor point depression most commonly measured by instruments)*
Chromatography	**Separation** of Mixtures into Individual Components **Based on** Specific Differences in **Physical Characteristics**
Electrophoresis	**Separation** of Molecules **Based on Ionic Charge & Size**
Potentiometry - pH meters, ion-selective electrodes *(ISE)*	Potential *(voltage)* **Difference Between Reference and Indicator Electrodes** when **No** Current is Passing through the Cell
Coulometry	**Titration** in which the Titrant is **Electrochemically** Generated
Amperometry	**Current** Flowing through an Electrochemical Cell when a **Constant Electric Potential** is Applied to the Electrodes

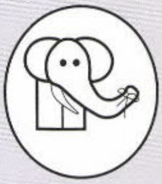
Hematology Instrumentation

METHODOLOGIES

1. Electronic impedance (*Coulter Principle*)
   a. Cell passing through electric current creates pulse proportional to cell size
   b. Used in cell counting/sizing and creating 3 part differential (*lymphs, monos, granulocytes*)
   c. RBCs and platelets counted in RBC bath
      ❖ *Differentiation based on size by using thresholds*
      ❖ *MCV measured directly (size of pulse proportional to cell size)*
   e. WBCs and nRBCs *(if present)* are counted in WBC bath *(RBCs are lysed)*

2. Optical Scatter (*Flow cytometry*)
   a. High intensity light of a single wavelength-LASER
   b. Used in cell analysis and differentiation
   c. WBC population identification based on differential light scatter (*forward vs. side*)
   d. Generates a 5 part differential

3. Cytochemical staining
   a. Peroxidase staining
   b. Aids in identification of WBC's based on degree of peroxidase activity



REMEMBER!

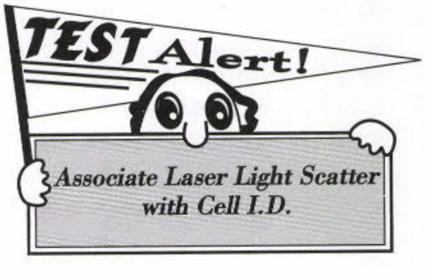
Light Scatter

FORWARD SCATTER

Cell Size

RIGHT ANGLE SCATTER

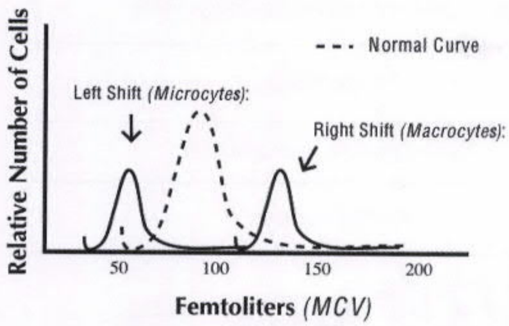
Interior Cell Structure (nuclei and granules)



Associate Laser Light Scatter with Cell I.D.

Histograms / Scattergrams

RED CELL HISTOGRAM

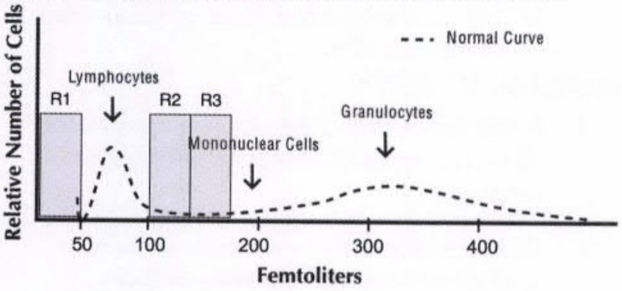


BIMODAL CURVE - 2 POPULATIONS OF RED CELLS

Example:

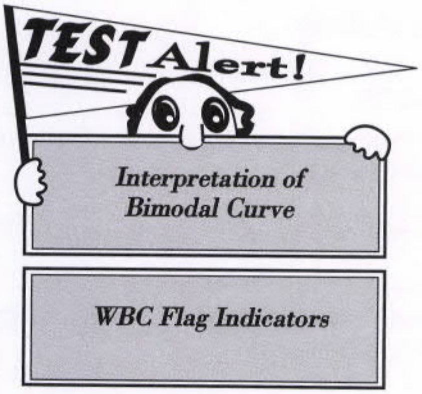
Left shift + Normal population = Fe deficiency anemia receiving transfusion

WHITE CELL- 3 PART DIFFERENTIAL HISTOGRAM



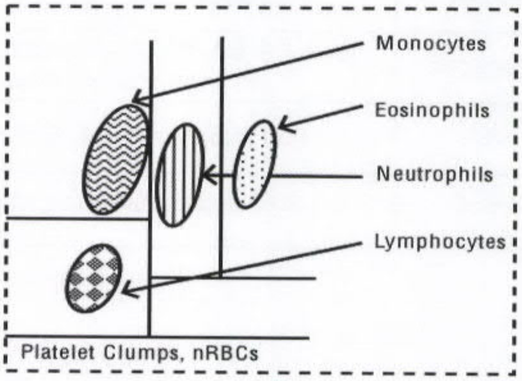
WBC FLAGGING INDICATIONS

FLAG	POSSIBLE CAUSES
R1	nRBCs, Platelet Clumps
R2	Blasts, Immature Granulocytes, Variant Lymphs
R3	Immature Granulocytes, ↑ Eos & Basos, Abnormal Cells


TEST Alert!
Interpretation of Bimodal Curve
WBC Flag Indicators

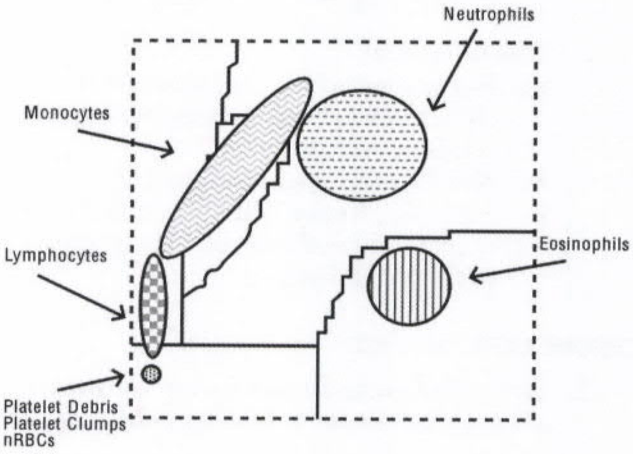
WBC SCATTERGRAM

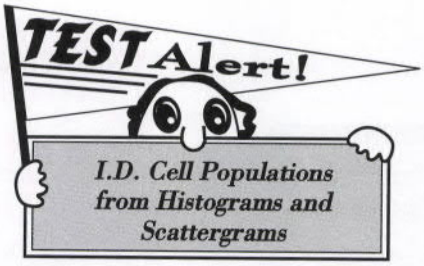
1. Beckman Coulter
   a. Impedence
   b. Volume, Conductivity, light Scatter (*VCS technology*)


Monocytes
Eosinophils
Neutrophils
Lymphocytes
Platelet Clumps, nRBCs

WBC/PEROXIDASE SCATTERGRAM

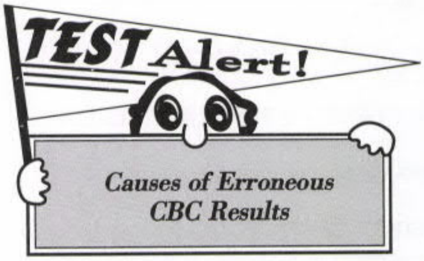
2. Seimens Advia
   a. Light Scatter
   b. Peroxidase staining


Neutrophils
Monocytes
Lymphocytes
Eosinophils
Platelet Debris
Platelet Clumps
nRBCs


TEST Alert!
I.D. Cell Populations from Histograms and Scattergrams

ERRONEOUS CBC RESULTS

PARAMETER	FALSE ↑	FALSE ↓
RBC	↑↑↑ WBC count, giant platelets	Schistocytes, microcytes, cold agglutinins
WBC	nRBCs, lyse resistant RBCs (HbS, HbC), giant platelets, platelet clumps, abnormal precipitant	Fragile WBCs
Platelet	Schistocytes, microcytes	Platelet clumps, giant platelets
Hemoglobin	Lipemia, ↑↑↑ WBC count, resisting Hb (S,C) abnormal globulins, icterus	Sulfhemoglobin
MCV	Cold agglutinins, ↑↑↑ WBC count	
MCHC	Cold agglutinins, lipemia, icterus	


TEST Alert!
Causes of Erroneous CBC Results

Coagulation Instrumentation

CLOT DETECTION METHODS

1. Electromechanical
   a. Clot causes decrease in movement of an iron ball in an electromagnetic field and triggers endpoint
   b. Clot times not affected by lipemic, hemolyzed or icteric samples

2. Photo-optical
   a. Formation of fibrin clot causes change in optical density which triggers end point
   b. Monitored by photodetector
   c. Lipemia, hyperbilirubinemia, extreme hemolysis may interfere with clot detection

CHROMOGENIC METHOD

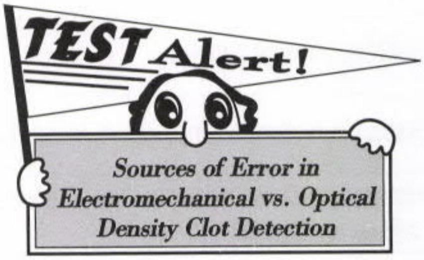
1. Activated coagulation factor (*enzyme*) cleaves substrate releasing color tag
2. Color change is directly related to factor concentration and is measured photometrically

IMMUNOLOGIC METHOD

1. Antibody coated latex microparticles directed against clotting protein or products

2. Antigen-antibody reaction causes agglutination and increased light absorbance

3. Detects presence of antigen but not functionality


TEST Alert!
Sources of Error in Electromechanical vs. Optical Density Clot Detection

Microbiology Instrumentation

SPECTROPHOTOMETRY

1. Principle (*see Chemistry/Immunology Instrumentation section*)

2. Infrared region - used in some blood culture methods to measure increases in CO_2 generated

FLUOROMETRY

1. Principle (*see Chemistry/Immunology Instrumentation section*)

2. Used in stains for microscopic exam

3. Used as labels in manual and automated systems for identification of organisms

CHROMATOGRAPHY

1. Used to determine type and relative amounts of organic acids produced by anaerobic organisms. Computerized data-base compares "tracing" from unknown to stored "tracings" of known organisms for identification

2. Used on cell wall extracts to identify organisms such as Legionella to species level

PULSE-FIELD GEL ELECTROPHORESIS (*PFGE*)

1. Evaluate nucleic acids for identification of sub-species typing

2. Important in epidemiology

CHEMILUMINESCENCE / NUCLEIC ACID PROBE

1. Principle
   a. Luminescent marker is attached to nucleic acid probe
   b. When probe hybridizes with target nucleic acid sequence, the bond with the luminescent marker is protected
   c. Bond between luminescent marker and unbound (*not hybridized*) probe is not protected and is easily hydrolyzed at 60° resulting in loss of chemiluminescent potential
   d. Luminescence is stimulated by a reagent that degrades the luminescent marker, resulting in a flash of light
   e. Light is measured using luminometer in relative light units (*RLU*) and is proportional to amount of bound (*hybridized*) probe

AUTOMATED BLOOD CULTURE

1. Determination of an increase in CO_2 in the culture bottle
   a. Increase in CO_2 results from increase in metabolism that occurs when organisms are growing

2. Increase in CO_2 is monitored at intervals
   a. Relative level of CO_2 is "growth index"
   b. Significant change is growth index results in culture flagged as positive

3. Increase in CO_2 can be measured by several methods
   a. Invasive
      - *Radiolabeled CO_2*
      - *Infrared spectrophotometry*
   b. Non-invasive
      - *pH indicators imbedded in bottles (as level of CO_2 increases, pH decreases)*
      - *Fluorescent sensors*
      - *Increase in pressure*

AUTOMATED BACTERIOLOGICAL IDENTIFICATION AND SUSCEPTIBILITY TESTING

1. Test panels consist of set of wells containing desiccated reagents for either biochemical or susceptibility testing

2. Panels of different types *(for organisms with different gram reactions or growth requirements)* are bar-coded for identification by instrument

3. Wells are inoculated with bacterial suspension which also rehydrates the wells

4. Control wells are used to detect contamination and potential for growth

5. Reactions are determined by several methods
   a. Turbidity
   b. Colorimetry
   c. Fluorescence

6. Identification is accomplished by comparison with computerized data base
   a. Most probable identification is reported at a predetermined confidence level
   b. Recommendations for additional tests may be made for organisms which cannot be identified at the confidence level

7. Some systems allow for rapid identification (*<6 hours*)

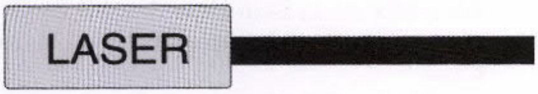
8. Most systems include incubator, automated reader, data terminal, and printer and can be linked to hospital information system

Urinalysis Instrumentation

1. Include individual strip readers, semi-automated, fully automated, and complete urinalysis workstations
2. Use reflectance photometry to determine each analyte concentration
3. Most commonly used analyzers:
   a. Clinitek *(Siemens)*
   b. Urisys *(Roche Diagnostics)*
   c. iQT 200 *(International Remote Imaging Systems)*
   d. *UF-1000i (Sysmex)*

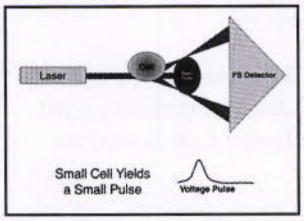
Flow Cytometry

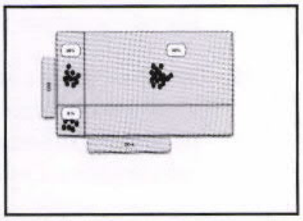
1. The measurement of cell properties using light in a fluid medium.
2. Major Components
   a. LASER
      - ❖ *Light Amplification by Stimulated Emission of Radiation*
      - ❖ *Argon gas LASER emitting light at 488 nm.*
   b. Beam blockers shield detectors
   c. Detectors capture photons of disturbed light and fluorescense signals from stains
   d. Flow cell contains cell stream
3. Principle
   a. Cells disrupt LASER beam to create light signals characteristic of cell size and complexity
   b. LASER excites fluorochromes attached to the cell in staining



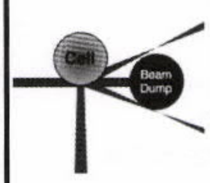
Monochromatic- single wavelength 488nm
Coherent- wave forms are in-phase, thus very bright
Directional- does not diverge, is easily directed to the cells

4. Forward Light Scatter
   a. depends on ***diffraction*** and ***refraction***.
   b. primarily measures the size of cells.





5. Side Scatter: (*90^o or orthogonal light scatter*):
   a. Detector is 90^o to laser beam
   b. Depends on ***reflection*** for signal
   c. Sensitive to size and granularity

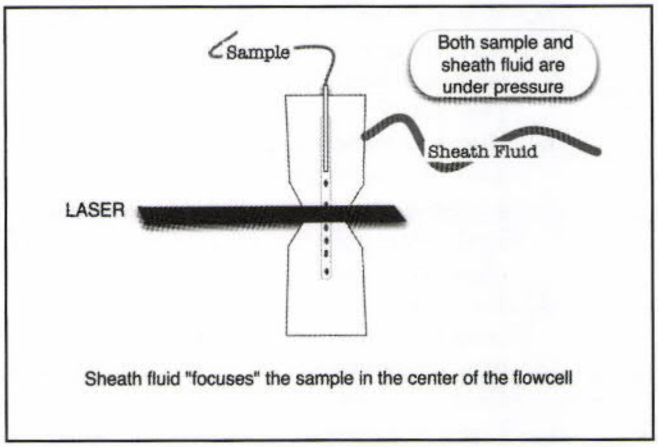


Diffraction: light bends when it hits the edge of the cell. Bigger edge= more photons.

Refraction: light bends when it enters the translucent cell and then enters the air again. Bigger cell= more photons.

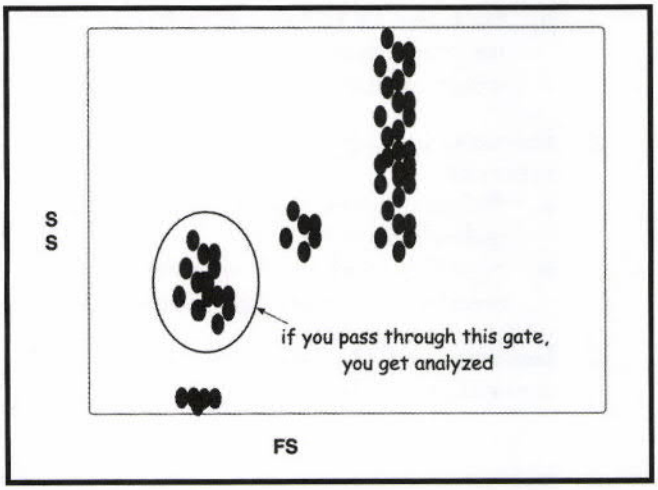
Reflection: light is bounced off and not absorbed. Granules= more reflection.

CELL STREAM IN THE FLOWCELL

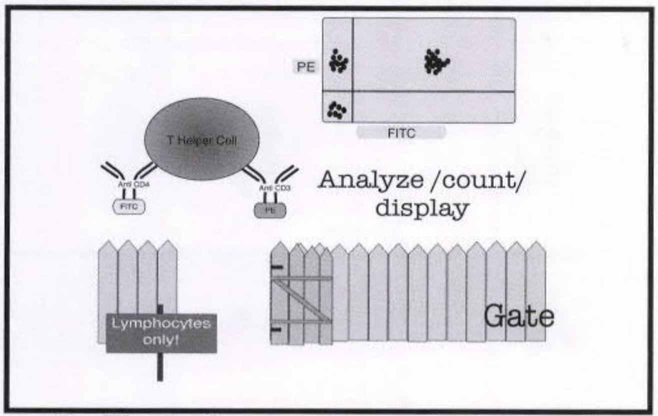


Sheath fluid "focuses" the sample in the center of the flowcell

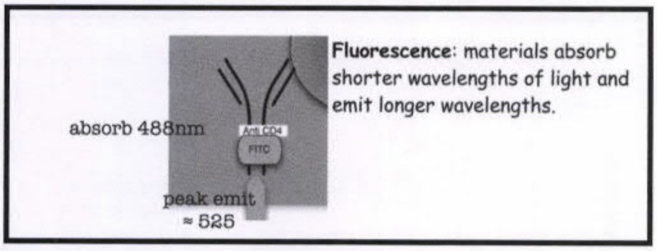
6. Separating Cells Electronically and Gating
   a. Lyse RBCs first
   b. Use FS and SS signals for 3 part WBC differential
   c. Populations gated (selected) for further study



Only cells that land in the gate region are analyzed for fluorescent properties. All other cells and events are ignored.



7. Fluorochromes
    a. Conjugated to monoclonal antibodies
    b. Direct fluorochromes
        - *Fluorescein isothiocyanate (FITC) green color (500-530nm)*
        - *Phycoerythrin (PE) yellow color (550-590nm) also called R-PE, RD1*
    c. Energy transfer conjugates / tandem dyes
        - *PE-texas red (ECD) orange color (600-630nm)*
        - *PE-cyanine5 red color (640-680nM) also called PC5*
        - *PE-cyanine7 infra red (710-770nm) also called PC7*

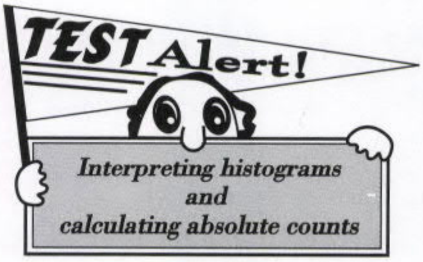


Fluorescence: materials absorb shorter wavelengths of light and emit longer wavelengths.

8. Antibodies
    a. For HIV immunophenotyping
        - *CD45- all leukocytes*
        - *CD4 - T helper lymphocytes*
        - *CD8 - T supressor lymphocytes*
        - *CD3 - all T lymphocytes*
        - *CD19- all B lymphocytes*
        - *CD16 and/ or CD56 - natural killer cells*
    b. Saturation Concentration
        - *Must be sufficient to bind all antigens*
        - *Must be verified as QC*

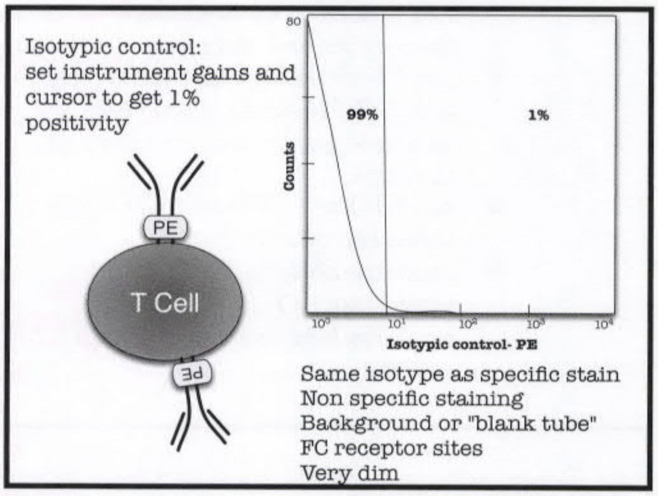
9. Lysis reagent(s)
    a. usually acid based
    b. eliminate rbcs

10. Analysis
    a. Single color
        - *one tube per antibody*
        - *gate lymphocytes with FS vs. SS histogram*
        - *requires isotypic control*
        - *replaced by multicolor analysis*
        - *does not meet immunophenotyping guidelines*



Interpreting histograms and calculating absolute counts

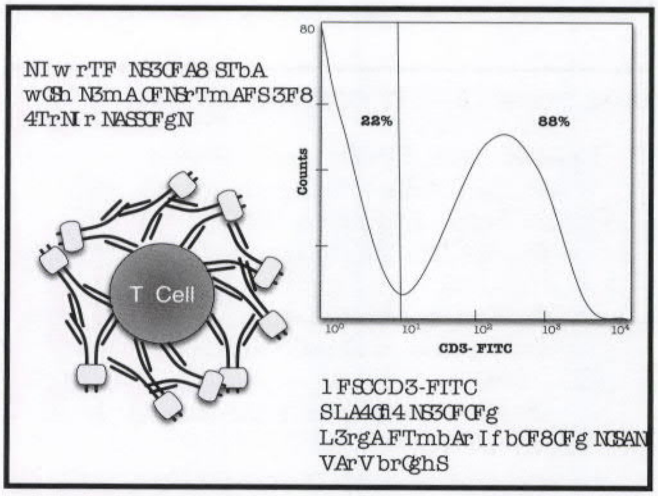
SET CURSOR USING ISOTYPIC CONTROL



Isotypic control:
set instrument gains and cursor to get 1% positivity

Same isotype as specific stain
Non specific staining
Background or "blank tube"
FC receptor sites
Very dim

DETERMINE THE PERCENT POSITIVE



NIwrTF NS3CFA8 STbA
wGh N3mACFNSrTmAFS3F8
4TrNI r NASSCFgN

1 FSCCD3-FITC
SLA4Gfi4 NS3CFCFg
L3rgAFTmbAr I f bCF8CFg NGSAN
VArVbrGghS

CALCULATING THE ABSOLUTE COUNT

Absolute count= (percent positive cells) x (percent lymphocytes) x (WBC)

Example: if CD3=88%, Lymphocyte %=45 and WBC=6500/mm^3

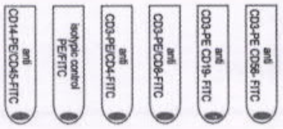
absolute CD3 count= .88 x .45 x 6,500

absolute CD3 count= 2574/mm^3

b. Two color analysis

- *mix two stains in one tube (e.g. FITC and PE)*
- *gate lymphocytes using FS vs. SS*
- *use "T-gating" mixtures (CD4-FITC/ CD3-PE) to exclude monocytes and debris*
- *use CD45 (stains all leukocytes) and CD 14 (stains moncytes) to measure purity and recovery of the gate*
- *use CD3 in CD19 and CD56+16 tubes for quality control*
- *measure all lymphocyte phenotypes (T,B, NK) and perform lymphosum as quality control*

Dual Color Analysis



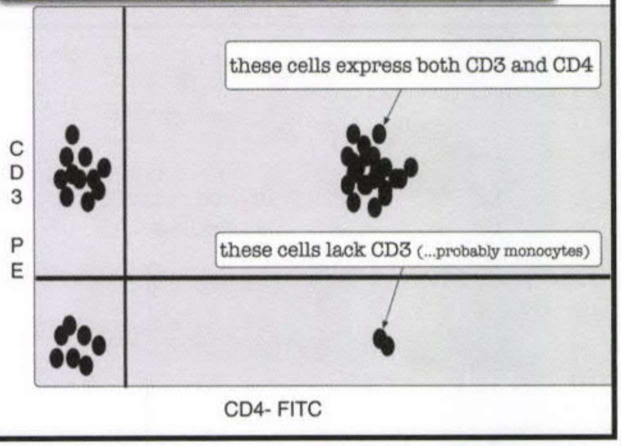
allows "T-gating" CD4 and CD8
CD3 controls tube to tube variables
allows for "lymphosum" calculation
CD14/CD45 measures purity and recovery

DUAL COLOR QUALITY CONTROL:

Lymphosum, Purity and Recovery

- %T cells + %B cells +%NK cells must add up to 100% or +/- 5%
- %CD3 should equal %CD4+%CD8 +/- 5
- Purity should equal or exceed 90%
- Recovery should equal or exceed 95%
- % CD3 in each tube should check +/- 3%

T-gating: To really be a CD4 positive lymphocyte you must also express the CD3 antigen

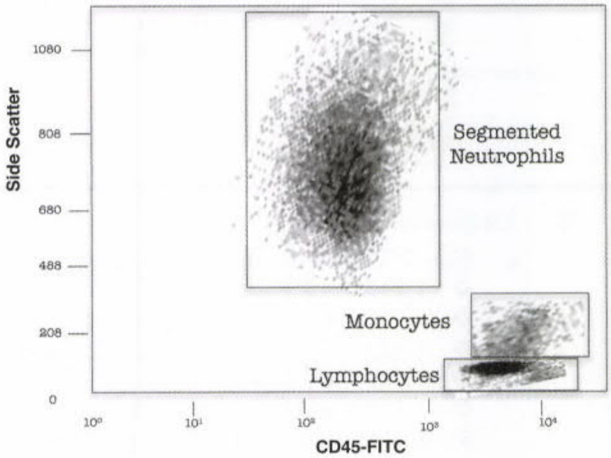


c. Three Color Analysis (all gate on CD45 vs side scatter)

Example three tube panel

1. CD3/CD4/CD45: measure CD3+CD4+ T-cells
2. CD3/CD8/CD45: measure CD3+CD8+ T-cells
3. CD3/CD19/CD45: measure CD3+ and CD19+ cells

note: CD3 serves as common lineage control and allows T-gating for CD4 and CD8



Gates are set on this histogram instead of FS vs SS when using three and four color systems.

Software packages automatically set optimum gate to maximize purity and recovery.

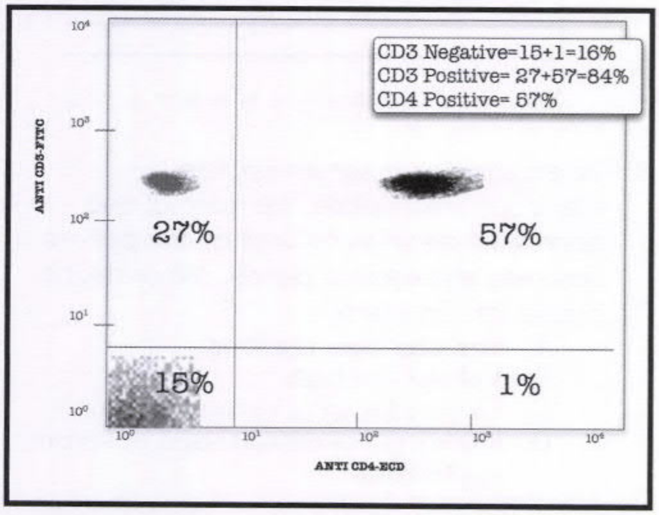
d. Four Color Analysis (all Gate on CD45 vs side scatter)

Example two tube panel

1. *CD45/CD4/CD8/CD3: measure CD3, CD8+ T-cells and CD4+ T-Cells*
2. *CD45/CD 56 (and /or)16/ CD19/CD3: measure CD56 (and or CD16), CD19+ cells and CD3+ Cells*

note: CD3 serves as common lineage control and allows T-gating for CD4 and CD8

11. Quadrant statistics
    a. The percentage of cells in each quadrant is calculated



12. Absolute counts
    a. Dual platform: use percentage positive from flow cytometer, percentage lymphocytes from automated differential and WBC count from hematology analyzer
    b. Single platform: use calibrated flow cytometer and /or add calibration beads to sample to determine absolute count directly

Example Calculation:

WBC = 10,500 / mm^3
Lymph % = 40%
CD4 % = 25%

10,500 X .40 = 4,200

4,200 X 25 = 1050/mm^3

The absolute CD4 count is 1050/mm^3

13. CD 4 Counts in HIV Monitoring
    a. In HIV infection, development of AIDS parallels the decline in absolute CD4+ cell numbers
    b. Normal range of CD4+ lymphocytes 450-1500/uL
    c. In HIV, start opportunistic infection prophylaxis if CD4+ cells drop below 500/uL.
    d. Clinical definition of AIDS= HIV infection and CD4+ cells below 200/uL

14. Common applications (other than HIV monitoring)
    a. Immunophenotying of leukemias and lymphomas
    b. Detection of fetal cells in maternal circulation
    c. Stem cell tranplantation support (using CD34)

LABORATORY OPERATIONS AND INSTRUMENTATION SAMPLE QUESTIONS

1. Bichromatic analysis is used in automation to
   - A. **Blank for background interference.**
   - B. **Decrease testing time.**
   - C. **Measure samples twice at timed intervals.**
   - D. **Verify wavelength calibration.**

2. The most common light source for spectrophotometry in the visible range is the
   - A. **Didymium lamp.**
   - B. **Deuterium lamp.**
   - C. **Hydrogen discharge lamp.**
   - D. **Tungsten lamp.**

3. What type of instrumentation is based on the principle of measuring energy emission that occurs when compounds absorb electromagnetic radiation, become excited and return to energy levels lower than their original energy levels?
   - A. **Atomic absorption spectrophotometry**
   - B. **Flame emission photometry**
   - C. **Fluorometry**
   - D. **Nephelometry**

4. The protein fraction that migrates most rapidly toward the anode is
   - A. Albumin.
   - B. Alpha-1.
   - C. Beta.
   - D. Gamma.

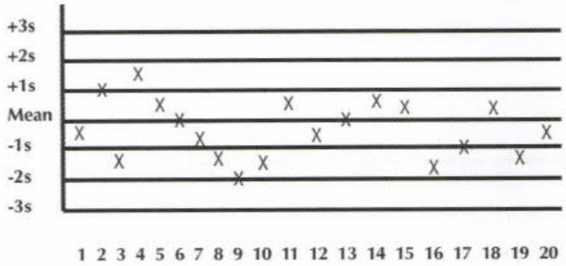
5. While performing an electrophoresis, it is noted that crescent-shaped bands develop. The next course of action is to repeat the electrophoresis and
   - A. **Use a new lot number of support media.**
   - B. **Reduce the application pressure.**
   - C. **NOT overload the sample.**
   - D. **Use serum instead of plasma.**

6. Kinetic assay of a serum enzyme gives the following data:

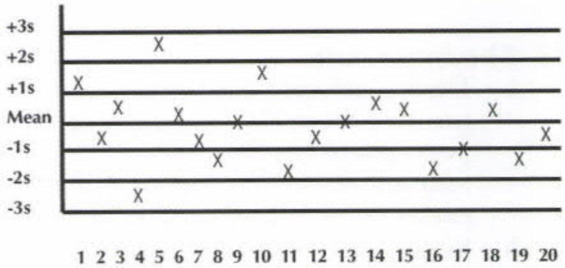
Time (min.)	Absorbance
0	0.005
1	0.035
2	0.075
3	0.135
4	0.195
5	0.215

   This assay demonstrates
   - A. Lag phase.
   - B. Linearity.
   - C. Substrate exhaustion.
   - D. Zero order kinetics.

7. How should a clinical laboratorian interpret the QC data shown below?
   - A. **Acceptable**
   - B. **Loss of precision**
   - C. **Shift**
   - D. **Trend**



8. When staining a serum protein electrophoresis plate, the normal and abnormal controls as well as the patient samples showed no bands. What might cause this problem?
   - A. **Staining time too long**
   - B. **Voltage too high**
   - C. **Electrodes were reversed**
   - D. **Excess pressure was used in serum application**



9. Using Westgard rules, evaluate the QC results above?
   - A. **10x rule broken, out-of-control**
   - B. **4(1s) rule broken, out-of-control**
   - C. **R4s rule broken, out-of-control**
   - D. **2(2s) rule broken, out-of-control**

10. An EIA procedure for HCG was performed. Low and high controls were run. Two standards (0 mIU/mL and 200 mIU/mL) were run in duplicate and results were calculated using average absorbances according to Beer's Law. Results on both controls were slightly higher than their respective control ranges. If one of the standard absorbances was eliminated, both controls would read near their respective control range means. Which standard absorbance was eliminated to accomplish this?
    A. 0 mIU/mL standard Absorbance 0.056
    B. 0 mIU/mL standard Absorbance 0.059
    C. 200 mIU/mL standard Absorbance 0.752
    D. 200 mIU/mL standard Absorbance 0.696

11. Which of the following disorders would be associated with a right shift in the red cell histogram?
    A. Thalassemia
    B. Fe deficiency anemia
    C. Pernicious anemia
    D. Aplastic anemia

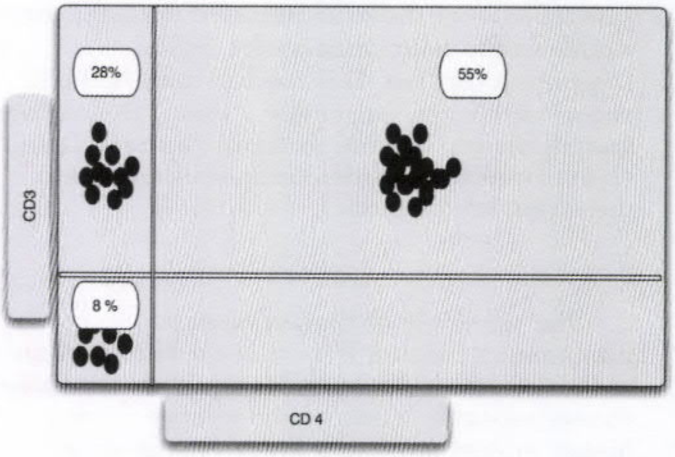
12. Which of the following may be a source of error in electromechanical clot detection methods?
    A. Lipemia
    B. Bilirubinemia
    C. Hypercoagulable sample
    D. Sample carryover

13. Which of the following is associated with a false increase in hemoglobin as well as the MCV and RBC count?
    A. Schistocytes
    B. Marked leukocytosis
    C. Microcytosis
    D. Lipemia

14. Using CD3/CD4 and CD3/CD8 mixtures in flow cytometry panels
    A. Is forbidden by accrediting agencies
    B. Ensures quality- only cells that co-express are counted positive
    C. Makes no sense since they mark different cells
    D. Is old technology and is not used

15. Given the follow data, calculate the absolute CD4 count.
    WBC= 5,500 / mm^3
    Lymph % = 20%
    CD4 %= 25%

    A. 275/ mm^3
    B. 2750/ mm^3
    C. 2250/ mm^3
    D. 225/ mm^3



16. Choose the correct statement that applies to the histogram (above)

    A. The CD4 is 83%
    B. The CD3 is 83%
    C. The NK cells are 8%
    D. The B cells are 28%

ANSWERS AND RATIONALE

1. A

In bichromatic analysis, absorbance is measured at two wavelengths. The baseline measurement is taken near the base of the peak, and the other is taken at the peak. The difference in absorption is proportional to concentration. This corrects for background interference (*e.g. lipemia, hemolysis, etc.*) in each sample.

2. D

The tungsten lamp produces a wavelength spectrum from the near infrared through the visible to the near ultra-violet region. Didymium (*option A*) is used to make filters, not as an energy source for a lamp. Deuterium and Hydrogen discharge lamps (*options B and C*) are used to provide continuous spectra in the ultraviolet region.

3. C

The principle of flame emission photometry (*option B*) is that excitation of an atom's electrons by heat energy from the flame causes unstable electrons to change from a higher energy state to a lower energy state. The principle of atomic absorption *(option A*) is the inverse of this, in which light is absorbed by ground state atoms. The principle of nephelometry (*option D*) is that the measured light is that scattered by particles in solution.

4. A

Albumin migrates the fastest because of its small size and negative charge. Separation of the major six bands, in order of migration, is pre-albumin, albumin, alpha-1, alpha-2, beta, and gamma. The gamma fraction migrates slightly toward the cathode because of electroendosmosis.

5. C

Crescent-shaped bands are usually caused by sample overloading or bent applicators. Option A may result in cracked or excessively dry material which may show distorted zones. Option B causes split bands. Option D will produce a fibrinogen peak which migrates between the gamma and beta fractions.

6. D

Plotting the absorbance vs time of the data presented shows a rapid increase between minutes 0 - 3 and a constant rate of reaction for minutes 3 - 5. Zero order kinetics occurs when a reaction follows the following process: 1) At the moment the enzyme and stustrate are mixed, the rate of reaction is zero but, 2) over time, the rate increases substantially and 3) remains constant for a period of time. Option A occurs when there is an initial period of time after the enzyme and substrate are mixed when the rate of reaction is zero. Option B occurs when the absorbance vs time increases proportionately throughout the assay. Option C is the point in time when the reaction rate begins to decrease due to lack of substrate for the enzyme.

7. D

Days 4 - 9 show consistently decreasing values. A trend occurs when a single control value increases or decreases for 6 consecutive days. (*Or, two control values increase or decrease for 3 consecutive days.*) Option B occurs when there is an increased distribution of control values beyond the + or - 2 S.D. limit. Option C is incorrect because a shift occurs when there is an abrupt change to a new mean.

8. B

If voltage is too high, proteins may be denatured (*option B*). If the plate remained in stain too long (*option A*), bands will stain but may be darker. If electrodes were reversed (*option C*), bands would appear but may not be well separated. If excess pressure were applied (*option D*), the medium may be cut and protein fractions may not migrate, but a band would be visible at the application point.

9. C

Days 4-5, R4s (*option C)* is correct because the values are 4s apart, one below the mean and one above the mean. 10x (*option A*) would require 10 values on the same side of the mean. 4(1s) (*option B*) requires 4 values to be outside 1s (all either above the mean or all below the mean. 2(2s) (*option D*) requires both values to be outside 2s on the same side of the mean.

10. C

Using the 200 mIU/mL standard with the higher absorbance would increase the slope of the curve, give lower control results, and lower results for the patient samples. Absorbances for the two 0 mIU/mL standards (*options A and B*) are too close to affect the slope of the curve. The 200 mIU/mL standard with the lower absorbance (*option D*) would decrease the slope and increase the concentrations of the controls and unknowns.

11. C

Options A and B are microcytic anemias and the red cell histogram would show a left shift.

In option D, the red cells are usually normocytic, normochromic.

12. D

Options A and B may interfere with photo-optical clot detection methods but will not affect electromechanical methods. Option C may result in an erroneously long clotting time using photo-optical methods if the clotting time is shorter than the guard interval.

13. B

Since WBCs are counted in the same bath as RBCs, markedly increased numbers of WBCs (*>100,000/µL)* will cause a false elevation of the RBC count as well as the MCV since they will be sized along with the RBCs. Also, a high WBC count will increase turbidity thereby increasing the hemoglobin value. Schistocytes are often too small to be counted even as red cells and their presence will not have any effect on the hemoglobin determination since they will be lysed along with the normal red cells. Microcytic red cells can be counted accurately, will cause a decreased MCV and do not falsely effect the hemoglobin measurement. Option D may cause a false elevation in the hemoglobin but will have no effect on the MCV or RBC count.

14. B

Since both CD4 and CD8 positive cells are T cells, they also express CD3 By pairing the antibodies we ensure that the cells are not monocytes (*which express CD4 but not CD3*). This technique is commonly called "T-gating". Pairing the markers ensures quality in other flow applications but this one is the best example.

15. A

5,500 X .2 = 1100

1100 X .25 = 275

16. B

The only true statement is B since the CD3 cells include those in quadrants 1 (*28%*) and 2 (*55%*). Adding these two percentages gives the total CD3 postive cells and allows for a common lineage quality control between tubes in a panel.

LABORATORY SAFETY & REGULATIONS

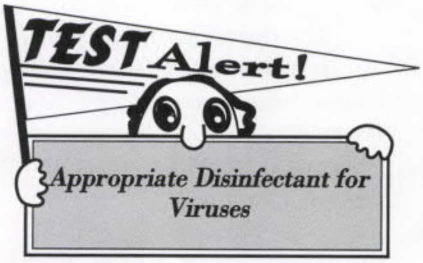
by Louann Lawrence *

BLOOD BORNE PATHOGENS

1. Universal (*standard*) precautions - All blood and body fluids are considered potentially infected with blood-borne pathogens

TEST Alert!
Concept of Universal Precautions

2. Examples of blood-borne pathogens:
   a. HIV - Human Immunodeficiency Virus
   b. HBV - Hepatitis B Virus
   c. HCV - Hepatitis C Virus
3. OSHA "Blood-Borne Pathogens" standard requires written "Exposure Control Plan"
4. Categories of exposure:
   a. Category 1 - exposed to blood and body fluids on a daily basis
   b. Category 2 - regularly exposed to blood and body fluids
   c. Category 3 - never exposed to blood and body fluids
5. Employers must offer hepatitis B vaccine at no cost to all personnel in Category 1 and Category 2
6. Identify tasks causing exposure to blood or body fluids
   a. Use engineering controls *(work shields, needle safety devices, pipeting devices, etc.)* to minimize risk of exposure
   b. Employers must provide PPE *(personal protective equipment)* at no cost when needed *(ex. gloves, lab coats and safety glasses)*
7. Good work practices
   a. Wash hands before leaving the lab, before using the biologic safety cabinet (*BSC*) and after removing gloves; first line of defense in infection control
   b. Do NOT mouth pipet
   c. Do NOT eat, drink, smoke, apply cosmetics, lip balm or contact lenses in clinical areas
   d. Do NOT bend, break, shear or recap used needles and syringes
   e. Clean up blood/body fluid spills immediately with 1:10 dilution of household bleach (*hypochlorite*) solution
   f. Clean counter tops, phones, keyboards on a regular basis with 1:10 dilution of household bleach (*hypochlorite*) solution
   g. Report all blood and body fluid exposures, document via incident report and have exposed person's blood tested as well as source patient's blood
   h. Employees have right to know lab results of source patient but must observe confidentiality
   i. Employees are entitled to medical consultation
   j. Use universal precautions with all reagents prepared from human blood or body fluids



8. Biological safety cabinet
   a. Facilitates safe manipulation of infectious material
   b. Reduces risk of exposure to personnel and laboratory area
   c. Directs airflow through high efficiency filter

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* original contribution by Mary Hebert

REMEMBER!
Biological Safety Cabinets

MUST monitor airflow

Use BSC with samples potentially containing pathogens transmitted by aerosolization.
Ex., *M. tuberculosis*, *C. imitus*, *F. tularensis* and *B. anthracis*

HAZARDOUS CHEMICALS

1. OSHA "Right to Know" (*Hazard Communication*) standard states employees have a right to know what hazardous chemicals they work with and how to protect themselves when using them.

2. Chemical Hygiene Plan
   a. Written plan stipulates what to do in case of a chemical spill, fire or exposure to chemicals in lab
   b. Criteria for control measures to reduce employee exposure
      - ❖ Engineering controls (*fume hoods, building ventilation systems, reinforced steel chemical storage cabinets, chemical hazard signs & labels*)
      - ❖ Use of personal protective equipment
      - ❖ Hygiene practices by employees (*frequent hand washing, avoidance of toxic fumes*)
   c. Fume hood *(used to protect workers from noxious or hazardous chemical reagents*) must be monitored periodically
   d. Employee training and the appointment of a chemical hygiene officer

3. Safety Data Sheets (*SDS*), formerly Material SDS (*MSDS*),- information provided by the chemical manufacturer stating risks of exposure, what to do if exposed, PPE required, and other important information, must be available for all chemicals used in lab

STOP NEVER store chemicals above head height.

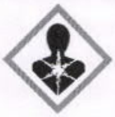
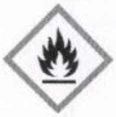
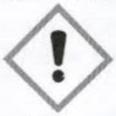
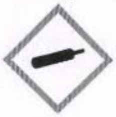
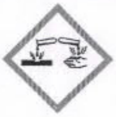
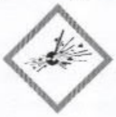
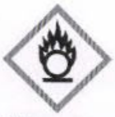
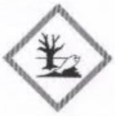
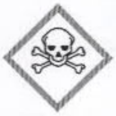
TEST Alert!
Appropriate Storage of Chemicals

4. Storage of chemicals
   a. Flammable solvents – store in flame cabinet; 1 gal or smaller containers may be kept under fume hood
   b. Corrosives – separate from other non-compatible chemicals (*alkali*) for storage
   c. Check SDS for specific storage requirements of chemicals

5. General information
   - ❖ *If a chemical is splashed in eye, go to nearest eye wash and wash eye for 15 minutes; seek medical attention*
   - ❖ *If a chemical is splashed on person or clothing, go to nearest body shower and rinse for 15 minutes; seek medical attention*
   - ❖ *Any chemicals considered carcinogenic should be used only while wearing gloves; consult SDS*
   - ❖ *Any chemical considered a respiratory health threat should be handled under a fume hood*

6. Revised Hazard Communication standard (*2012*)
   a. Definitions of hazard changed to provide specific criteria for classification of health and physical hazards
   b. Chemical manufacturers required to include label with a signal word (*pictogram*) and hazard statement for each hazard class and category using the United Nations' global chemical labeling system, the Globally Harmonized System (*GHS*). (*See pictograms next page*)
   c. Safety Data Sheets (*SDS*) will be standardized to contain a specified 16-section format.
   d. Employees should be trained on the new pictograms by end of 2013

HCS Pictograms and Hazards

Health Hazard  • Carcinogen • Mutagenicity • Reproductive Toxicity • Respiratory Sensitizer • Target Organ Toxicity • Aspiration Toxicity	**Flame**  • Flammables • Pyrophorics • Self-Heating • Emits Flammable Gas • Self-Reactives • Organic Peroxides	**Exclamation Mark**  • Irritant (skin and eye) • Skin Sensitizer • Acute Toxicity (harmful) • Narcotic Effects • Respiratory Tract Irritant • Hazardous to Ozone Layer (Non-Mandatory)
Gas Cylinder  • Gases Under Pressure	**Corrosion**  • Skin Corrosion/ Burns • Eye Damage • Corrosive to Metals	**Exploding Bomb**  • Explosives • Self-Reactives • Organic Peroxides
Flame Over Circle  • Oxidizers	**Environment** (Non-Mandatory)  • Aquatic Toxicity	**Skull and Crossbones**  • Acute Toxicity (fatal or toxic)

Source: https://www.osha.gov/Publications/OSHA3491QuickCardPictogram.pdf

REMEMBER!

Chemical Spills

C *ontain the spill*
L *eave the area*
E *mergency: eye wash, shower & medical care*
A *ccess SDS*
N *otify supervisor*

Purpose of SDS

Meaning of HCS Symbols

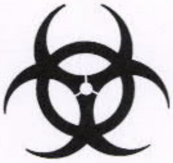
WASTE DISPOSAL

1. Types
    a. Hazardous waste - solid waste or mixture of solid wastes which may pose a threat to human health or the environment when improperly handled
    b. Infectious waste - equipment, utensils or substances that may harbor or transmit pathogenic organisms from individuals who may have a communicable disease
    c. Medical waste - any solid, semisolid or liquid waste generated in diagnosis, treatment or immunization of humans or animals in research or production or testing of biologics

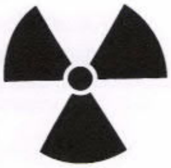
2. Identified by orange or red seamless plastic bags labeled with the biohazard symbol

TEST Alert!
Recognize Safety Symbols

Biohazard



Radiation



3. Sharps containers must be rigid, puncture-proof and leakproof

4. Treat infectious or medical wastes by incineration or autoclaving *(public trash collection NOT suitable for disposal of raw infectious waste)*

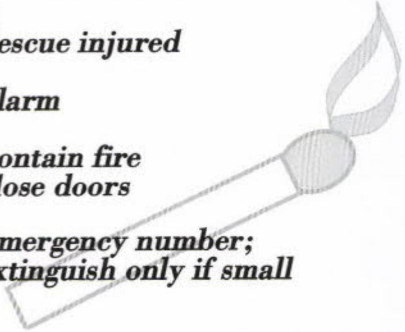
5. Secured storage area for infectious material to prevent accidents in handling

RADIATION SAFETY

1. Dispose of all radioactive material in appropriate labeled container
2. If working with large amounts of radioactive material, use protective shielding between worker and material
3. Wear proper PPE and adhere to safe work practices
4. Report any exposure to radioactive material and seek medical attention
5. Radiation monitoring
    a. Film badge or dosimeter
    b. Exposure limits (*maximum permissible dose equivalents - 5000 mrem/yr; whole body)*
    c. Wipe test - laboratory work surfaces wiped with absorbent material (*wipe*); radiation contained in each wipe counted

REMEMBER!

Fire Safety



***R**escue injured*

***A**larm*

***C**ontain fire*
***C**lose doors*

***E**mergency number;*
***E**xtinguish only if small*

FIRE SAFETY

1. What to do
    a. Alert staff
    b. Rescue any injured
    c. Pull nearest fire alarm
    d. Contain fire - close doors
    e. Call institution emergency number
    f. Find nearest fire extinguisher; only attempt to put out fire if it is small

2. How to use a fire extinguisher
    a. Use appropriate class of extinguisher
        - ❖ *Class A - wood and paper fires*
        - ❖ *Class B - flammable liquid fires*
        - ❖ *Class C - electrical fires*
        - ❖ *Class D - reactive metals*
    b. Most fire extinguishers can be used on A, B, and C fires
    c. Halon Gas
        - ❖ *Heavier than O_2; displaces O_2 near fire which extinguishes fire*
        - ❖ *Will not harm lab equipment*
    d. To operate fire extinguisher, remember PASS
        Pull pin
        Aim nozzle at bottom of fire
        Squeeze trigger slowly
        Sweep nozzle

NEVER use water on flammable liquids or electrical fires.

ELECTRICAL SAFETY

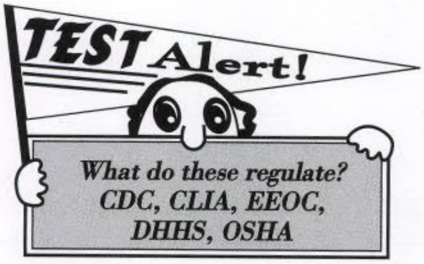
1. Lock out/tag out malfunctioning electrical or mechanical equipment until serviced.
2. Report any small shock (*or "tingle"*); unplug and tag equipment until serviced.
3. Replace all frayed wires and plugs
4. All equipment must be grounded (*preferably with 3-pronged plugs*)
5. If a severely shocked person cannot let go of instrument, unplug it (*without touching it*) or knock person loose with nonconductive material, such as wood
6. If the shock victim stops breathing, perform CPR

COMPRESSED GAS

1. Store cylinders vertically chained to wall or with anti-tip collar
2. Keep away from sources of heat, electricity or flammable liquids
3. Keep valve covered with protective cap until immediately before use
4. Monitor pressure daily when gas is in use
5. Permanent label identifying contents is required

REGULATORY AGENCIES

1. U.S. Department of Health and Human Services *(DHHS)* – oversees FDA, CMS, and CDC
2. U.S. Food and Drug Administration *(FDA)* – regulates use of numerous products including medical devices and blood products; approves new laboratory tests, technology and instruments
3. Centers for Medicare and Medicaid Services *(CMS)* – federal agency responsible for administration of Clinical Laboratory Improvement Act of 1988 *(CLIA 88)*, Medicare / Medicaid services, and contains the Office of the Inspector General *(OIG)*
4. Centers For Disease Control and Prevention *(CDC)* – national advisory agency that develops disease prevention standards and guidelines and promotes environmental health; does not have regulatory or enforcement authority
5. Office of the Inspector General *(OIG)* – governmental agency that investigates Medicare / Medicaid billing fraud and abuse
    a. Compliance program – voluntary plan to assure laboratory's adherence to regulations established by CMS to prevent fraud and abuse
6. Occupational Safety and Health Administration *(OSHA)*- federal agency that regulates employee safety in the workplace
7. Department of Transportation *(DOT)* - administers the Hazardous Materials Standard which requires specific procedures for shipping of biohazardous materials and employee training

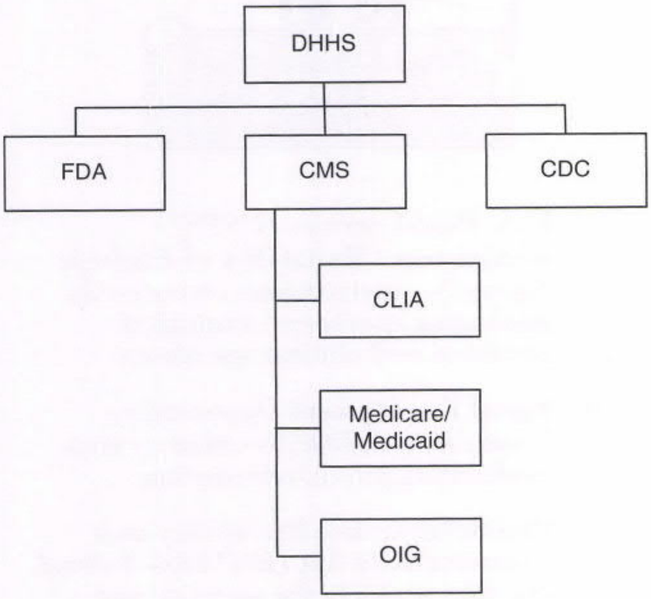


What do these regulate?
CDC, CLIA, EEOC, DHHS, OSHA

8. U.S. Postal Service *(USPS)* - administers "Mailability of Etiologic Agents" - –instructions on correctly packaging specimens, biological products and clinical specimens
9. Equal Employment Opportunity Commisson *(EEOC)* – enforces laws prohibiting job discrimination
10. Health Insurance Portability and Accountability Act *(HIPAA)* – federal law that protects the security and confidentiality of healthcare information
    a. Protected Health Information *(PHI)* – individually identifiable information *(electronic, paper or oral)* relating to a patient's health condition or payment for healthcare

CLINICAL LABORATORY IMPROVEMENT ACT OF 1988 (CLIA 88)

1. Federal law that established minimum standards for laboratory practice and quality; applies to all laboratories in U.S. performing tests on humans for the purpose of medical treatment
2. Purpose – to ensure accuracy, reliability and timeliness of patient test results regardless of where test was performed
3. Extent of regulation based upon complexity level of testing *(waived, moderately complex, and highly complex)* and risk of harm to patient when incorrect results are reported
4. Provisions include
   a. Quality control and quality assurance
   b. Use of proficiency tests
   c. Personnel qualifications
   d. Performance improvement
   e. Patient test management
5. All labs must be certified every 2 years



VOLUNTARY ACCREDITING AGENCIES

1. The Joint Commission – inspects and accredits hospitals and other health care facilities
2. College of American Pathologists *(CAP)* – voluntary accrediting agency for clinical laboratories
3. American Association of Blood Banks *(AABB)* – voluntary accrediting agency for blood banks
4. Commission for Office Laboratory Accreditation (*COLA*) – voluntary accrediting agency for physician office laboratories and community hospitals
5. Deemed status – alternative to direct federal oversight granted to the above agencies to enforce CLIA regulations

TEST Alert!
Name 3 Voluntary
Accrediting Agencies

SAFETY SAMPLE QUESTIONS

1. The concept that laboratory personnel should treat all blood and body fluids as capable of transmitting infectious diseases is known as
   - A. Infection control
   - B. Quality control
   - C. Safe practice standards
   - D. Universal precautions

2. Of the following, which would be considered personal protective equipment?
   - A. Biologic safety cabinet
   - B. Latex gloves
   - C. Sharps container
   - D. Work shields

3. What type of fire extinguisher would be best to use on computer equipment?
   - A. CO_2
   - B. Foam
   - C. Halon
   - D. Water

4. A clinical laboratorian's first response to a formalin spill in the laboratory is to
   - A. Call 911
   - B. Consult the SDS
   - C. Evacuate all personnel from the area
   - D. Notify the supervisor

5. Which of the following is a voluntary accrediting agency?
   - A. CDC
   - B. DHHS
   - C. The Joint Commission
   - D. OSHA

6. If an employee suspects that the employer is violating safety standards, which agency should be notified?
   - A. OSHA
   - B. OIG
   - C. CDC
   - D. CLIA

ANSWERS AND RATIONALE

1. D

The Centers for Disease Control and the Clinical and Laboratory Standards Institute (*CLSI*) have developed a laboratory personnel protection system that is comprised of engineering controls, personal protective equipment and work practice controls. These are designed to guard workers from the potential of exposure to blood-borne pathogens. Option A is a hospital promoted concept primarily focusing on washing hands as a way to prevent hospital spread infections. Option B is those procedures that ensure the accuracy of test results and includes but is not limited to testing control materials with known value ranges, equipment maintenance and proficiency testing of personnel. Option C sounds good but doesn't mean much in terms of a specific concept.

2. B

Options A, C and D are examples of engineering controls.

3. C

Halon is heavier than air and will displace the O_2 putting out the fire. It will NOT damage equipment, which is a major advantage.

4. C

Evacuate personnel first. Then notify the supervisor and consult the SDS for appropriate information.

5. C

The Joint Commission is the only agency listed that is voluntary and that accredits health care organizations

6. A

The Occupational Safety and Health Administration (*OSHA*) oversees standards that regulate safety in the workplace, including the Bloodborne Pathogen and Chemical Hazard Standards. An employee may request an OSHA inspection if violations are suspected.

Congratulations on completing your review!!!

We hope this book has been helpful in preparing you for your courses and exams. Please send suggestions or ideas for future editions to:

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